HEREDITY, VARIATION, AND THE APPEARANCE OF DIVERSITIES DURING THE VEGETATIVE REPRODUCTION OF *ARCELLA DENTATA*¹

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	95
The problem, p. 95; advantages of <i>Arcella</i> for genetic studies, p. 96; characters studied, p. 97; cultural methods, p. 97; fission in <i>Arcella</i> , p. 99; parent and offspring, p. 99; counting generations, p. 100; preservation of specimens, p. 101; length of life and number of progeny, p. 101; computations, p. 102.	
Variations in spine number and diameter of shell in a "wild" population.....	102
Heredity and variation in spine number in families derived from single specimens by vegetative reproduction	105
Permanent diversities in spine number among the descendants of a single specimen produced by vegetative reproduction	109
Data before selection was begun, p. 111; first selection period, p. 112; second selection period, p. 114; third selection period, p. 115; fourth selection period, p. 115; fifth selection period, p. 116; sixth selection period, p. 117; conclusion, p. 119; non-selection periods, p. 120; selection within the high line, p. 122; selection within the low line, p. 123.	
Permanent diversities in diameter of shell among the descendants of a single specimen produced by vegetative reproduction	124
a. Diversities in diameter of shell between the high and low lines of family 58	124
b. Diversities in diameter of shell between the high and low branches of the low line of family 58.....	126
Diversities in diameter of shell and in spine number due to sudden large variations ("mutations")	127
Branch EF, p. 127; branch E421, p. 129; branch EM, p. 129; branch ED, p. 132.	
Empty shells	139
DISCUSSION	142
SUMMARY	148
LITERATURE CITED	150

INTRODUCTION

The problem

In the vegetative reproduction of rhizopod Protozoa conditions for the study of heredity, variation, and the method of evolution are more

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simple than in any other animals. The body of *Arcella* is a single cell, which is as simple as any animal organism it is possible to obtain for such studies. During vegetative reproduction the two offspring at each division consist each of one-half of the parent. There are thus no heritable variations due to amphimixis, and if changes occur that are of evolutionary significance they may be easily recognized. During the past four years several reports have appeared, notably those of MIDDLETON (1915), JENNINGS (1916) and ROOT (1918), that cast some doubt upon the conclusions of earlier workers, that heritable diversities do not occur within a "pure line," that is, that the genotype is constant. Work of this sort is crucial, since it tests one of the requirements of evolution, i.e., that heritable diversities should appear that are not due to the mixture of factors from several individuals. The present contribution adds one more species of Protozoa in which there is an apparent inconstancy of the genotype during vegetative reproduction.

Advantages of Arcella for genetic studies

Arcella dentata was chosen as the material for this investigation because it possesses an unusually large number of favorable characteristics (fig. 1.)

FIGURE 1.—A, aboral view of a typical specimen of *Arcella dentata* with 11 spines and a diameter of 116 microns. Imbedded in the cytoplasm (dotted) are 2 nuclei, a number of contractile vacuoles, and smaller food material. $\times 310$.

B, aboral view of a specimen just after division. This specimen had 13 spines and measured 138 microns in diameter. $\times 310$.

(1) It multiplies vegetatively and rapidly so that many generations may be obtained in a short period of time.

(2) It possesses definite morphological characters that are easily measured and not modified by growth.

(3) Environmental factors have no influence upon these characters after division is completed.

(4) Its characters are heritable but variable.

(5) The shell may be preserved easily, thus furnishing a permanent record.

(6) It is large enough to be conspicuous when placed under a Greenough binocular.

(7) The young are much lighter in color than their parents and hence there is no danger of confusing parent and offspring.

(8) The examination of living specimens is simple, because they attach themselves by their pseudopodia to the substratum and move very slowly.

(9) The almost transparent shell reveals the nuclei, chromidia, contractile vacuoles and other bodies within the living animal—especially in young specimens.

(10) Individuals will withstand severe operations and halves and even quarters will survive and reproduce provided they contain a nucleus.

Characters studied

The characters reported upon in this paper are (1) the number of spines, and (2) the diameter of the shell, measured between the bases of the spines. Other characteristics available for study are the size and shape of the spines (figure 5), the rate of fission, and the color of the shell.

Cultural methods

Arcella dentata, according to LEIDY (1879) and others, is rare when compared with *A. vulgaris* and *A. discoides*; nevertheless, an abundant supply was obtained from the lily pads in a pond on the campus of the JOHNS HOPKINS UNIVERSITY at Homewood, Baltimore.

The cultural methods used were similar to those employed by JENNINGS (1916) in his work on *Diffugia*. Water plants from nearby ponds were brought into the laboratory and the diatoms, algae, and ooze covering them were washed off by shaking in a bottle. The coarser materials were strained out and then the water containing the finer particles was transferred with a pipette to concavities in hollow-ground slides. As the work progressed, it was found that the *Arcellas* were not so apt to become covered by sediment if the culture slides were prepared from three to twelve hours before needed. During this time the particles sink

to the bottom, forming a fine film upon which the animals creep about and feed. It was also discovered that the Arcellas were more active and multiplied more rapidly if the pond water was diluted with an equal amount of distilled water. Thus in each cavity were placed two drops of the pond water containing food particles and two drops of distilled water. The brownish Arcellas are quite distinct against the grayish film of sediment on the bottom of such a culture, and there is no danger of contamination due to the inclusion of "wild" specimens that might occur in the food material.

New cultures were prepared, on the average, every three days, and the Arcellas were transferred to them with a capillary pipette. The cultures were examined almost every day and the young were removed to separate slides soon after they were produced. The number of the individual was then written with pencil on the rough surface of the slides. The cultures were not covered with cover glasses but were kept from drying by placing them in moist chambers consisting of large stender dishes. Each of these dishes accommodated eleven slides, or twenty-two specimens.

Specimens were easily found in the cultures with the help of a Greenough binocular and were then more closely examined under a compound microscope with the use of a water immersion objective.

For keeping pedigree records the system used by JENNINGS was adopted. This system was described by JENNINGS (1916, p. 415) as follows:

"For keeping pedigrees, records are kept in card catalogues, a card being devoted to each individual. The system may be illustrated as follows: The original 'wild' individual is given a number—say 21. Its first progeny is called 21.1, its second 21.2, etc. The first progeny of 21.1 is 21.1.1, its next 21.1.2, etc. Thus in later generations we have cumbrous labels such as 326.1.4.2.2.3.2.1.2.2.2.1.1.2. These labels must be written on the slide and its corresponding card. On the card is written a brief description of the individual, including the number of spines, and any peculiarity that will distinguish it from its progeny. Whenever an individual reproduces, that fact, with its date, and the number of spines of the progeny, is entered on the card of the parent, and a new card is written for the progeny. The long labels become troublesome, but each gives the full pedigree and relationship of the individual, and with the card records of all, the entire history may be reconstructed"

It was found desirable in certain cases to substitute for some of the longer labels one or more letters, such as **A**, **B**, **ED**, **SS**, etc.

Fission in Arcella

The process of fission in *Arcella* has not been described in detail and some of the stages were not observed during the course of this work. When ready to divide enough cytoplasm is protruded from the mouth of the shell to secrete a new shell; this cytoplasm lies against the substratum beneath the parent shell and thus the process of shell formation can not be observed from above. It seems probable that the spines of the shell are secreted around pseudopodia which extend out from this cytoplasmic mass. A number of specimens were examined after the new shell had been secreted but before division had taken place. In every case most of the cytoplasm was in the parent shell and only a small plug of cytoplasm extended into the new shell as shown in side view in figure 2. Cytoplasm then gradually flowed into the new shell until each shell contained approximately an equal amount. The cytoplasmic strand extend-

FIGURE 2.—Side views of division stages of *Arcella dentata*. The parent is at the left; the offspring at the right. The extent of the protoplasm is indicated by the dotted lines. The intervals between the stages are as follows: between A and B, 7 minutes; B and C, 3 minutes; C and D, 10 minutes. $\times 207$.

ing between the mouth openings of the two shells then grew thinner and finally broke and the parent and offspring moved apart sideways. There was no immediate swelling of the cytoplasm within the shells, which were therefore only about half full, as shown in figure 1B.

Parent and offspring

The method of division of *Arcella* with respect to the shell, presents several difficulties not encountered in the study of shellless Protozoa. When it divides, the protoplasm apparently divides into two equal parts, i.e., the living substance of the parent passes over into that of the two daughters, but whereas one of the daughters is provided with a new shell, the other continues to live in the parental shell. Throughout this

paper I have referred to the latter as the parent and the former as the offspring. If a specimen has 12 spines, this number must be due to two factors, (1) the hereditary constitution of the protoplasm of its parent and (2) the environmental conditions during or preceding the formation of its shell. It is thus safe to conclude that the number of spines it possesses is a good index of its hereditary constitution, since it contains one-half of the parental protoplasm that secreted the shell and the environment was kept relatively constant. However, we have found that the young often vary from the parent in spine number and the problem thus arises as to whether the protoplasm of a 12-spined parent that has just given rise to a 10-spined offspring really corresponds to the shell in which it is confined or is a "square peg in a round hole." On this account a number of correlation tables were made up from data of the immediate or first progeny of each parent. When these tables were compared with those in which later offspring were included, it was found that the results were so nearly identical that no further attention was paid to this apparent difficulty. It seems probable that heritable diversities do actually appear among the progeny of a single individual, but the diversities are so small and the number of offspring belonging to other than the first division that were recorded were so few that very little difference results from their inclusion in correlation tables.

Counting generations

A second difficulty that arises because of the persistence of one-half of the parental protoplasm in the parental shell at the time of division is the counting of generations. This is of some importance since we measure the "permanency" of a characteristic in part by the number of generations through which it persists. Should the second offspring from a "parent" be considered as a member of the f_1 or of the f_2 generation, and should the parent after each fission be considered as belonging to the same generation as its last offspring? The accompanying parts of a pedigree (figure 3, **A**, **B**) show that such a parent may be considered a member of either generation, but really belongs to the f_2 generation. For convenience, however, all numbers of generations mentioned in this paper, unless otherwise indicated, have been counted according to the first method. It is a very simple matter to determine the number of the generations according to either method from the system of labelling employed. For example the specimen designated by the numerals 58.I.I.I.4.2.I.I.I.I.3.I.3.I.2.4 belongs to the fifteenth generation according to the first method, this answer being found by counting the number of nu-

FIGURE 3.—Diagrams illustrating two methods of counting generations in *Arcella*. The diagram at the left, A, shows that the original parent with 10 spines gave rise successively to 2 offspring with 13 and 9 spines, respectively, and that each of these in turn gave rise to 2 offspring, the one with 13 spines to a first with 10 spines and a second with 11 spines, and the one with 9 spines to 2 successive offspring with 11 spines each.

In the diagram at the right, B, the parent after division is in each case shown as belonging to the same generation as the offspring.

merals after the family number, 58; or belongs to the twenty-seventh generation according to the second method, this answer being found by adding the same numerals together.

Preservation of specimens

It was found impossible in the time available, to preserve all the specimens, but a large proportion of them were saved for further study as this was found to be necessary. This was accomplished by transferring each individual to a separate drop of glycerine on a glass slide. One slide will hold without crowding about eight isolated specimens. The labels were written with India ink on the slides which were then placed in upright boxes where they keep for months without drying.

Length of life and number of progeny

No attempt was made to determine how many offspring may be produced by one parent nor how long a single parent will live. However, in looking over the records, it was found that specimen 58.1.1.1.1.2.1.1.1.1.1.2.2.1.1.1.2.1 was kept from December 20, 1917, to January 25, 1918, during which period it produced 18 offspring, and specimen 58.1.1.1.4.2.1.1.1.1.3.1.1.1.1 was kept from December 7, 1917, to January 24, 1918, and gave rise to 21 progeny. There seems to be no reason why

these specimens might not have continued to live and reproduce indefinitely.

Computations

In the tables presented in this paper the coefficients of correlation were determined by means of the formulae used by JENNINGS (1916, pp. 416-417). The actual pedigrees, however, are perhaps better evidence than correlation tables of what has really occurred, and as many parts of pedigrees have been included as seemed advisable.

The specimens that form the basis of this report were obtained during a period of 174 days. The average number of generations studied was 69 and the average length of a generation was 2.5 days. In all, measurements of 6474 specimens are recorded. Of these 5557 belonged to the single family No. 58; 746 were secured from 70 small families; and 171 were collected directly from the pond.

VARIATIONS IN SPINE NUMBER AND DIAMETER OF SHELL IN A "WILD" POPULATION

The principal problem that this investigation was designed to solve is, Do variations in heritable characteristics that may occur in the progeny of a single specimen reproducing vegetatively lead to temporary or permanent diversities that would account for the diversities present in a "wild" population?

We must, of course, determine first what the variations are in such a "wild" population. Accordingly a large number of specimens of *Arcella dentata* were collected from various parts of the pond where they were

FIGURE 4.—Three specimens from a "wild" population of *Arcella dentata*: A was the smallest specimen found, possessing 7 spines and a diameter of 73 microns, B was the largest, with 17 spines and a diameter of 150 microns, C was in the modal class and near the mean, with 11 spines and a diameter of 116 microns. $\times 207$.

FIGURE 5.—Some variations in the sizes and shapes of the spines of *Arcella dentata*.
 X 420.

present in great abundance. These were found to vary in size, and in number and length of spines. Some of these variations are indicated in figures 4 and 5. The extremes with respect to spine number were 7 and 17; and those with respect to diameter of the shell were 73 microns and 150 microns. No study was made of the variations in the shape or size of the spines, but such variations were incidentally noted and some of them are shown in figure 5.

TABLE I

Correlation table for spine number and diameter of the shell in a "wild" population of *Arcella dentata* collected from a pond at Homewood, Baltimore, on Sept. 20, 1917. The unit of measurement is 4.3 microns. Correlation, $.325 \pm .060$.

	Spine number							
	7	8	9	10	11	12	13	
23	1							1
24			1	2	1			4
25			2	12	4	1	1	20
26			3	12	10	3		28
27			4	12	7	2		25
28			3	4	5	5		17
29				2	1			3
30								0
31								0
32								0
33						1	1	2
	1	0	13	44	28	12	2	100

Table I shows the variations in spine number and diameter of the shells of 100 wild specimens taken at random from a large series collected on September 20, 1917, from the pond at Homewood and figures 6 and 7 give the same data in the form of curves. As regards spines, the mode is 10 and the mean 10.42, and for diameter the mode is 26 units and the mean 26.51 units. Specimens were found with spine numbers

of 8, 14, 15, 16 and 17, but these did not happen to appear in this sample of 100. A coefficient of correlation of $.325 \pm .060$ shows that there is a significant relation between spine number and diameter in these or-

FIG. 6

FIGURE 6.—Curve showing the distribution of spine numbers of 100 "wild" specimens of *Arcella dentata* collected on Sept. 20, 1917, from a pond at Homewood, Baltimore. The ordinates are numbers of specimens and the abscissae numbers of spines.

FIG. 7

FIGURE 7.—Curve showing the distribution of diameters of the 100 specimens recorded in figure 6. The ordinates are numbers of specimens and the abscissae diameters. The unit of measurement is 4.3 microns.

ganisms. Frequently irregular specimens were encountered (figure 12) principally among those with a low spine number; pedigree work indicates that many of these irregularities are due to environmental factors. They undoubtedly account for the high mean diameter of 26.46 of the group of 13 specimens with 9 spines shown in table 2. With the ex-

TABLE 2

Mean diameters of 100 "wild" specimens of *Arcella dentata* of given spine numbers. The unit of measurement for diameter is 4.3 microns.

Number of specimens	Spine number	Mean diameter
1	7	23.00
13	9	26.46
44	10	26.22
28	11	26.50
12	12	27.50
2	13	29.00

ception of this group, the mean diameter of the specimens measured increases gradually with the increase in spine number, exhibiting a marked correlation between these two characteristics.

Conclusion

The variations, therefore, in a random sample of 100 "wild" specimens are from 7 to 13 in spine number and from 23 to 33 units in diameter of shell, each unit being equal to 4.3 microns, and there is a marked correlation between the number of spines and the diameter of the shell.

HEREDITY AND VARIATION IN SPINE NUMBER IN FAMILIES DERIVED FROM SINGLE SPECIMENS BY VEGETATIVE REPRODUCTION

The next questions to be answered are, What kinds of variations occur during vegetative reproduction and to what degree are they inherited? Are heritable characteristics similar or different among families ("pure lines") descended from different "wild" specimens? To what degree are these heritable characteristics correlated in each family? Are there in nature a number of diverse families with regard to a given characteristic and are the several measurable characteristics correlated or independent in the various families? How many diverse families are there?

One hundred and forty-nine living specimens were selected from collections of wild animals, mostly taken on September 21 and 22, and November 23, 1917, and isolated on culture slides. These specimens were selected on the basis of diameter and spine number so that a beginning might be made with animals differing widely in these characters, as well as with those nearer the modal condition. Of the 149 specimens, 59 gave rise to small families and 12 to larger families. All of these, except one, either died out or were killed off because they could not be taken care of; the one family, number 58, was kept, being selected for further work. Tables 3 to 20 include only data regarding spine number.

Table 3 shows the distribution of the variations in number of spines within twelve of the larger families obtained. These are arranged according to the mean spine number beginning with the lowest mean. The range is from 10.40 to 14.07. A large interval occurs between the mean 11.21 of family 41 and that of 13.71 of family 62. Probably a larger number of families would supply means that would fill up this gap. Possibly, however, families 62, 109, and 83 should be considered as belong-

TABLE 3
Distribution of the variations in number of spines within twelve families.

Number of family	Number of spines										Total number of specimens	Mean spine number	Coefficient of correlation	
	7	8	9	10	11	12	13	14	15	16				
18	1	1	4	8	11	5					30	10.40	.004 ± .123	
85		2	5	12	14	6					39	10.43	— .158 ± .105	
80		1	3	11	9	2	2				28	10.50	.013 ± .013	
30			22	11	9	5	11				58	10.51	.279 ± .082	
79		4	2	11	10	11					38	10.57	.032 ± .001	
81		1	1	8	17	5	1				33	10.81	.085 ± .117	
17		2	17	31	39	45	12	3			149	11.04	.062 ± .055	
59			3	2	7	6	2				20	11.10	.039 ± .151	
41			4		1	22	8	4	2		41	11.21	— .176 ± .102	
62				1	2	2	2	4	11	9	4	35	13.71	.725 ± .054
109						1	2	4	11	10		28	13.96	.183 ± .128
83							5	7	13	10	5	40	14.07	.312 ± .093
											539			

ing to a distinct race. In only three families, 30, 62, and 83, is there any evidence of correlation between the spine numbers of parents and offspring.

Figure 8 gives the curves for four families separately and for twelve

FIGURE 8.—Curves showing the distribution of the variations in spine number within four small families and for the total, T, of 539 specimens belonging to twelve families. The number with which each curve is marked is the designation of the family. The numbers of specimens in each family are shown in table 3. The ordinates are percentages; the abscissae, spine numbers.

families combined. Figures 9 and 10 are parts of the pedigrees of two of these families, showing quite clearly the great difference between them in spine number.

FIG. 9

FIGURE 10

FIGURE 9.—Part of the pedigree of family 80 showing the number of spines of specimens belonging to the first 3 generations. The progenitor of this family (ABN; see fig. 12, A) was not normal and its abnormality was not inherited. It was selected for breeding purposes in order to determine whether or not abnormalities in *Arcella dentata* are inherited.

FIGURE 10.—Part of the pedigree of family 83 showing the number of spines of specimens belonging to the first three generations. This pedigree should be compared with that shown in figure 9. The abnormal progenitor of family 83 (see fig. 12, B) gave rise to normal offspring.

In all correlation tables the results of every fission were tabulated. Thus, when a specimen with 12 spines gave rise to one with 10 spines, the latter was recorded opposite number 10 in the parent column 12. Each offspring is counted but once as an offspring.

Table 4 presents the correlation for the inheritance of spines in the 70 small families taken together. This table consists of two principal sets of data; (1) the center of the table is occupied largely by offspring descended from specimens collected mostly on September 21 and 22, 1917. These have a mean spine number of a fraction less than 11.00. (2) The lower right-hand corner contains principally the descendants

TABLE 4
Correlation table for parents and offspring with respect to spine number within 70 small families. Correlation, $.626 \pm .015$.

		Parents																
		7	8	9	10	11	12	13	14	15	16	17						
Progeny	7																	1
	8		2	1	1	3	4	1							12			
	9	1	1	12	11	13	9	1							48			
	10		9	12	28	40	36	10		1					136			
	11		9	16	37	44	44	9	4	1					164			
	12		2	5	17	38	25	25	7	4					123			
	13		2	3	3	9	17	24	11	9					78			
	14		1			1	4	24	20	29	18	6			103			
	15						2	11	14	11	10	12	1		61			
	16							2	1	4	8	4			19			
17									1					1				
		1	26	49	98	153	173	105	68	50	22	1	746					

of very large specimens with many spines collected on November 23, 1917. This table shows that a high correlation ($.626 \pm .015$) exists between the spine numbers of parents and progeny in this large population containing progeny derived from 70 "wild" specimens.

In table 5 is given the mean spine number for the 746 offspring produced by parents with different numbers of spines within the 70 families studied. It is clear that the spine number of the progeny approaches more closely that of the immediate parents than it does the mean of the race.

Among the small families that were studied were several whose curves

TABLE 5
Mean spine numbers of 746 progeny of parents of given spine numbers within 70 small families.

Number of specimens	Spine number of parents	Mean spine number of progeny
1	7	9.00
26	8	10.69
49	9	10.42
98	10	10.72
153	11	11.00
173	12	11.66
105	13	12.75
68	14	13.72
50	15	14.12
22	16	14.90
1	17	15.00

of distribution of spine numbers almost coincide. This, of course, might happen with families derived from specimens taken from widely separated localities, but in this case it seems probable that, since the specimens were all collected from a rather small pond and some of them from a single leaf, they were closely related and hence gave rise to families similar in the distribution of spine number.

Conclusion

Variations in spine number occur among the descendants of a single specimen during vegetative reproduction, and these variations are in part inherited. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number. There is a marked correlation between parent and offspring with respect to spine number in three of the families (30, 62, and 83). The small differences between certain of the means, e.g., between those of the first five families listed in table 3, namely, 10.40, 10.43, 10.50, 10.51, and 10.57, indicate that, with respect to spine number, the heritable diversities between the different families may be extremely small and that a vast number of families exhibiting such heritable diversities may occur in a "wild" population. The large gap between the means 11.21 and 13.71 of families 41 and 62 would probably be bridged if a larger number of families were obtained, but this may represent a difference that might be considered of racial magnitude.

PERMANENT DIVERSITIES IN SPINE NUMBER AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

At the end of 39 days from the time the rearing of the Arcellas was begun, their numbers had increased to such an extent that it was necessary to eliminate all but one family. The latter, family 58, was retained for the purpose of determining the degree of inheritance and the extent of diversities in a single large family, and to answer the following questions: Do variations that may appear in heritable characteristics in the descendants of a single specimen reproducing vegetatively lead to temporary or permanent diversities? May such diversities account for the heritable differences that appear between families obtained from "wild" specimens? In what way are such diversities produced?

The family derived from specimen 58 was saved for this work since its mean spine number was near the average of the species and its members seemed more viable than those of the other families. For convenience and also for the purpose of checking up environmental influence,

Non-selection period; 11 days; 7 gen.

Three selection periods; 11 days; 11 generations. Non-selection periods; 11 days; 7 generations.

Four non-selection periods,
64 days; 22 generations.

Six selection periods.
64 days; 22 generations.

Before
selection,
39 days;
7 gen.

FIGURE 11.—Diagram showing the results of selection within the single family 58 for diversities in spine number. Each triangle or rectangle represents a period. The triangles are intended to indicate a gradual increase from one or a few selected individuals at the point at the left to a large number at the right. The rectangles indicate that the number of specimens was kept the same throughout the period. Within each triangle or rectangle is given the number of specimens studied during that period, and above each is given the mean spine number for the period. The upper series of triangles and rectangles give the data for the high line, and the lower series the data for the low line. Between the two series are the differences in mean spine number between the high and low lines. During the periods 2, 3 and 4, of the first series of selection periods, the high and low lines were made up of three branches each and labelled A, B, C, and D, E, F, respectively. The means for the three branches taken together during these periods are given in parentheses above the triangles in the high line and below the triangles in the low line.

the experiment was divided into a number of periods. The length of these periods was regulated so that enough progeny were obtained in each to give significant means. The periods were also determined in part by the fact that only a few hundred specimens can be taken care of by one investigator and when this limit has been reached a selection must be made. It may be noted at this place that several sudden large variations occurred in several of the periods, but the specimens exhibiting them, and their descendants are not included in the data presented in this part of the investigation but are given later under a separate heading. The data for the periods are given in tables 6, 14, and 18 to 21, and are indicated in the diagram on page 110 (figure 11), reference to which will make the following account clear.

Data before selection was begun

The progenitor of family 58 (figure 13, **A**) was collected on September 22. It had 12 spines. Because of faulty cultural methods it died after dividing only once, and a number of the early members of the family died from the same cause (figure 14). Later, specimens were very seldom lost, although a few died, some dried because of the evapo-

FIGURE 12.—Three abnormal specimens: **A** was the progenitor of family 80, and **B** of family 83; **C** apparently lacks one or two spines because of some mechanical obstruction in the environment. $\times 207$.

FIGURE 13.—Three specimens belonging to family 58. **A**, the progenitor of the entire family; **B**, a typical member of the low line; **C**, a typical member of the high line. $\times 207$.

FIGURE 14.—The first part of the pedigree of family 58.

ration of the culture water, and several were lost during their transference from one culture to another. The first period was the longest and lasted for 39 days. During this time 198 offspring were produced with a mean spine number of 10.87. Table 7 gives the data for these 198 specimens. This table shows no evidence of correlation between parents and offspring, the coefficient of correlation being $-.027 \pm .048$.

First selection period

On November 1 selection was begun. All specimens with 12 or more spines were selected as a basis for the high line and all those with 10 or less for the low line. These and all of their offspring were kept. The specimens selected for both the low and high lines were placed in the same moist chambers; were given the same proportions of food and water; and were all changed at the same time. Environmental condi-

tions were thus almost exactly alike for all the specimens at any particular time during the course of the work. At the end of 9 days this period was closed. The diagram on page 110 and table 6 show that the 37 progeny of the high line had a mean spine number of 11.05, and the

TABLE 6

Family 58. Results of selection for number of spines during selection periods 1 to 6. (Nov. 1, 1917, to Jan. 3, 1918.)

Period	Number of progeny	Correlation	High line		Low line		Difference
			Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (9 days)	78	.060 $\pm$.076	37	11.05	41	11.12	-.07
2 (25 days)	273	.220 $\pm$.039	94	11.22	179	10.72	.50
3 (9 days)	245	.186 $\pm$.042	42	11.09	203	10.69	.40
4 (8 days)	271	.185 $\pm$.040	88	11.21	183	10.73	.48
5 (7 days)	162	.403 $\pm$.044	64	11.32	98	10.48	.84
6 (6 days)	163	.512 $\pm$.039	92	11.48	71	10.32	1.16
64 days	1192					Mean difference .55	

TABLE 7

Family 58. Correlation table for parents and offspring with respect to spine number before selection was begun. Correlation, $-.027 \pm .048$.

		Parents				
		9	10	11	12	13
Progeny	9	3	4	1		8
	10	9	19	15	2	45
	11	5	12	21	25	6
	12	1	16	19	14	6
	13	8	5	7		20
		6	48	68	62	14
		198				

TABLE 8

Family 58. Correlation table for parents and offspring with respect to spine number in the first selection period (Nov. 1 to Nov. 9, 1917).

Correlation .060 $\pm$.076.

		Parents					
		8	9	10	11	12	13
Progeny	8					1	1
	9					1	1
	10	4	8	1	7	2	22
	11	4	10	1	10	2	27
	12	3	5	1	9	2	20
	13		3		3	1	7
		11	26	3	31	7	78

41 progeny of the low line had a mean spine number of 11.12. The diversity obtained was thus in the wrong direction, the mean spine number of the low line being actually greater by .07 than that of the high line. Table 8 indicates an entire absence of correlation during this period.

This result may have been due to the small number of offspring used, but later work makes it seem more probable that the selection was not successful, because high- and low-spined specimens were picked out regardless of the condition of their immediate and more distant ancestors,—a method that JENNINGS (1916) has shown to be inefficient under similar conditions in *Diffugia corona*.

Second selection period

The method of selection was changed at this time (November 10) and specimens with 12 or 13 spines that had parents, grandparents or other close relations with a high spine number were selected as progenitors of the high line, and similarly, specimens with 10 or less spines whose near relatives possessed low spine numbers, were selected as progenitors of the low line; that is, selection was based on past performance. All parents and offspring were kept during this period which extended over 25 days, from November 10 to December 4. Difficulty was encountered in the rearing of the Arcellas at this time. Many of them ceased to divide and all of the specimens became entangled in the food material to such an extent that they had to be extricated with needles. Large numbers died and the entire family seemed doomed, when a simple remedy was discovered. This was the dilution of the pond water, with which the cultures were made up, with an equal amount of distilled water. This procedure was suggested to me by the fact that Dr. VERNON LYNCH of the JOHNS HOPKINS MEDICAL SCHOOL, had previously found that the addition of distilled water to his cultures was of benefit in the rearing of Amebas. Division immediately began again and no more trouble was experienced throughout the entire winter and spring seasons.

During the second selection period the 94 progeny in the high line possessed a mean spine number of 11.22, and the 179 progeny of the low line, a mean spine number of 10.72, or a divergence of .50. The mean spine number of the high line was thus .35 above the mean of the family before selection was begun and that of the low line was .15 below this mean. Table 9 shows the degree of correlation of parent and offspring during this period.

It was found that at the end of the second selection period all progeny in the high line were descended from three specimens, and similarly, all

those in the low line were descended from three specimens. The branches to which these specimens had given rise were from this time on designated as **A**, **B**, and **C** in the high line, and **D**, **E**, and **F**, in the low line, and the data for these six branches are indicated in the diagram on page 110. It is worthy of note that the mean spine number of each of the

TABLE 9

Family 58. Correlation table for parents and offspring with respect to spine number in the second selection period (Nov. 10 to Dec. 4, 1917).

Correlation .220 $\pm$.039.

		Parents					
		8	9	10	11	12	13
Progeny	8	1					1
	9		5	4	5		14
	10	1	34	23	9	3	70
	11		7	40	43	20	2
	12	1	11	28	22	5	67
	13		1	3	4	1	9
		9	92	101	60	11	273

three subfamilies in the high line is greater than that of any of the three subfamilies in the low line, and that the means of all six subfamilies differ slightly from one another. Too much emphasis should not be attached to this but it suggests the possibility that these six subfamilies may represent six permanent heritable diversities in spine number.

Third selection period

At the end of the second selection period a few specimens were selected from each of these six branches on the basis of past performance and all parents and progeny were kept until the end of the period (9 days). As shown by the diagram on page 110, the three branches of the high line did not increase as rapidly as did those of the low line. During this period the mean spine number of the 42 offspring in the high line was 11.09, and of the 203 offspring in the low line, 10.69, giving a divergence of .40.

As during the second selection period, the mean spine number of each of the three branches of the high line is greater than that of any of the three branches of the low line. Table 10 shows the degree of correlation between parent and offspring during this period.

Fourth selection period

At the end of the third selection period a few specimens were selected from each of the three branches of the high line and from each of the

TABLE 10

Family 58. Correlation table for parents and offspring with respect to spine number in the third selection period (Dec. 5 to Dec. 13, 1917).

Correlation $.186 \pm .042$.

		Parents										
		8	9	10	11	12	13					
Progeny	8	2						2				
	9		5	13	4			22				
	10	1	8	38	13	9		69				
	11	2	13	44	20	18		101				
	12	1	6	18	4	11	5	45				
	13			4	1	1		6				
							4	32	119	42	39	9

nated after being recorded and all specimens that persisted in giving rise to low-spined offspring were likewise eliminated; in a similar way all offspring in the low line with more than 10 spines were eliminated, and also all specimens that produced several high-spined progeny. All progeny were, of course, included in the data before they and their parents were eliminated. This method of selection was most effective. The mean spine number of the high line rose to 11.32, that of the low line decreased to 10.48, and the divergence increased to .84. Table 12 shows the degree of correlation between parents and offspring during this period, and figures 15 and 16 give parts of the pedigrees of the two lines at this time.

Sixth selection period

Selection at the beginning and during this period was carried on in

FIGURE 15.—Part of the pedigree of the high line of family 58 during the fifth selection period.

FIGURE 16.—Part of the pedigree of the low line of family 58 during the fifth selection period.

FIGURE 17.—Curves showing the distribution of the variation in spine number within the high and low lines of family 58 during the non-selection periods. The ordinates are percentages; and abscissae, spine numbers; H= the high line; L= the low line.

TABLE 12

Family 58. Correlation table for parents and offspring with respect to spine number in the fifth selection period (Dec. 22 to Dec. 28, 1917).

Correlation $.403 \pm .044$.

		Parents						
		8	9	10	11	12	13	14
Progeny	8		1	2	1			4
	9	1	6	1		1		9
	10	2	9	14	6	7	3	41
	11	1	3	20	18	28	5	75
	12	1	1	5	5	10	3	26
	13				1	5		6
	14					1		1
		5	20	42	31	52	11	162

the same way as during the fifth period. A rise in mean spine number to 11.48 in the high line, and a decrease to 10.32 in the low line gave a divergence of 1.16. Table 13 shows the degree of correlation between parents and offspring during this period.

Selection was discontinued at the end of the sixth period. The mean spine number had increased in the high line from 10.87, which was the

TABLE 13

Family 58. Correlation table for parents and offspring with respect to spine number in the sixth selection period (Dec. 29, 1917 to Jan. 3, 1918).

Correlation $.512 \pm .039$.

		Parents						
		8	9	10	11	12	13	14
Progeny	8		2	2				4
	9	3	2	2	1		1	9
	10	2	6	18	1	9	1	37
	11		5	15	4	19	12	56
	12		3	5	3	20	12	43
	13				2	7	3	13
	14						1	1
		5	18	42	11	55	30	163

mean of the family before selection was begun, to 11.48, a difference of .61, and had decreased in the low line from 10.87 to 10.32, a difference of .55. The average difference between the means of the high line and the low line during these six selection periods was .55.

Conclusion

It has thus been demonstrated that by means of selection, two lines can be obtained from a single specimen reproducing vegetatively, that differ in measurable, heritable characteristics. This difference may seem

slight but it is constant not only for the high and low lines, but also for the separate branches (**A, B, C** and **D, E, F**) into which these lines were divided, and hence is significant. The question now arises, Is this divergence temporary or permanent?

Non-selection periods

To test the permanency of the divergence obtained during the six selection periods of 64 days, a series of non-selection periods was inaugurated. Because it is impossible for one investigator to take care of more than a few hundred specimens at one time, the following method was used during the first three non-selection periods. Twenty-two representative specimens (the number that can be accommodated in one moist chamber) were taken from the high line, and 22 from the low line. Whenever these divided, the parent was eliminated and the offspring kept. Thus the number of specimens was kept down but no selection was practiced. Only immediate progeny obtained during the first three non-selection periods were thus recorded, but during the fourth period, several offspring from a single parent were included. The data obtained during the four non-selection periods are presented in figure 11, and in tables 14, 15 and 16. As the diagram on page 110 and table 14 show, there was an immediate decrease in the difference between the means of the high and low lines from 1.16 to .94 during the first non-selection period and to .07 during the second non-selection period. During the third period the difference increased to .41.

At the end of the third non-selection period, the high and low lines were allowed to expand; that is, *all* parents and offspring were kept. This was continued for 15 days and a very large number was obtained in

TABLE 14
Family 58. Spine numbers during the four non-selection periods (Jan 3 to Feb. 6, 1918).

Period	Number of progeny	High line		Low line		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (6 days)	186	104	11.90	82	10.96	.94
2 (7 days)	183	102	11.91	81	11.84	.07
3 (7 days)	188	87	11.42	101	11.01	.41
4 (15 days)	768	306	11.60	462	11.26	.43
35 days	1325	Mean difference .46				

TABLE 15

Family 58. High line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (588) progeny, 11.94. Correlation, $.170 \pm .027$.

	Parents								
	8	9	10	11	12	13	14	15	
Progeny 9			4		1				5
10		2	10	18	17	6	2		55
11		4	28	32	71	20	5		160
12		2	30	62	114	43	6		257
13		1	11	17	40	25	3	1	98
14			2	1	5	3	1		12
15						1			1
	9	85	130	249	97	17	1		588

TABLE 16

Family 58. Low line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (737) progeny, 11.28. Correlation, $.197 \pm .024$.

	Parents								
	8	9	10	11	12	13	14	15	
Progeny 8				1					1
9		1	6	4	4	2			17
10	2	8	30	47	27	7			121
11		8	48	135	92	19	1		303
12		2	50	82	72	21		2	229
13			6	20	19	17			62
14				1	1	1			3
15					1				1
	2	19	141	290	215	67	1	2	737

each line; 306 in the high line and 462 in the low. Again a difference between the two lines appeared—this time of .43. Since the divergence between the high and low lines persisted throughout the 4 periods of non-selection, representing 18 generations, the conclusion was reached that a permanent divergence had been obtained.

It will be noticed that the mean spine number increased in both the high and low lines during the first non-selection period. Thus that of the high line during the sixth selection period was 11.48 and in the first non-selection period was 11.90, and that of the low line during the sixth selection period was 10.32 and in the first non-selection period 10.96. The mean spine number of the high line increased only by .01 during the second non-selection period; whereas that of the low line increased by .88. A decrease in both lines occurred in the third non-selection period, and an increase again in the fourth non-selection period. Several other

lines were being propagated at the same time and in these similar increases and decreases were noted during these periods. One of these lines consisted of specimens derived from a large *Arcella* collected on December 27, 1917, thus showing that these changes in spine number were not restricted to descendants of specimen 58. A similar increase in spine number was noted by JENNINGS (1916, p. 489) in *Diffugia corona*. The cause of these increases and decreases is not known but their appearance in all lines at the same time points to some environmental factor, probably the composition of the water or food. Some of the data for certain days during these periods (table 17) indicate very clearly the extent of these increases and decreases.

TABLE 17

Family 58. Table showing distribution of the spine numbers of progeny of high and low lines on three dates. On Dec. 31, the distribution was almost "normal" for the two lines. On Jan. 15 the spine number had increased in both, but more noticeably in the low line. On Jan. 18 a reduction in the number of spines in both lines to near the normal is indicated; and on Jan. 26, an increase again in both lines.

Date	Line	Spine number							Total number of progeny	Mean spine number
		8	9	10	11	12	13	14		
Dec. 31	High			1	13	17	5		36	11.72
	Low	3	2	18	8	5			36	10.27
Jan. 15	High			1	4	5	4		14	11.85
	Low				4	5	1		10	11.70
Jan. 18	High			2	3	7			12	11.41
	Low			4	4	3			11	10.91
Jan. 26	High				3	4	3	1	11	12.18
	Low			1	3	1	1		6	11.66

Divergences similar to those obtained in family 58 are no doubt occurring in nature at all times, and probably lead to greater differences there than those recorded above. Such differences as exhibited by families 80 and 83 (see figures 9 and 10) might have arisen in this way, and, while the means of certain branches from these lines might approach each other and even coincide, the lines as a whole would remain distinctly different, each possessing a different mean. (See figure 27.)

Selection within the high line

The problem that was next undertaken was to determine whether high and low lines could be obtained from each of the high and low lines al-

ready secured. Accordingly a few specimens were selected from the high line as progenitors of a new high line, and a few were selected from the high line as progenitors of a new low line. These selections were made, as formerly, on the basis of past performance, and all parents and progeny were kept during a period of six days. The results as in-

TABLE 18

Family 58. High line. Results of selection for high and low numbers of spines (Feb. 8 to Feb. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
I (6 days)	595	300	11.47	295	11.48	-.01

indicated in the diagram on page 110 and in table 18 show that the mean spine number of the 300 specimens in the high line was 11.47 and that of the 295 specimens in the low line was 11.48—a difference of only .01. This slight result and lack of time necessitated the discontinuance of this experiment so that selection could be continued in the low line, but the data obtained from the latter leave no doubt that further work would have been successful.

Selection within the low line

Specimens with a high spine number and those with a low spine number were selected on the basis of past performance from those of the low line that were living at the end of the non-selection periods. These were reared during three selection periods of 4, 9 and 6 days respectively. Selection was practiced at the end of each period and also during the periods. As shown in the diagram on page 110 and in table 19 a divergence was obtained of .19, .37, and .34 respectively during the three selection periods. A non-selection period was then inaugurated lasting for 11 days (table 20). At the beginning of this period a representative group of specimens was selected in each line. A divergence of .44 resulted. Since the data thus obtained seemed to prove that two lines differing in their heritable characteristics had been obtained from the low line, the work with these lines was discontinued.

It seems probable also that divergences of a similar sort would be

TABLE 19

Family 58. Low line. Results of selection for high and low numbers of spines (Feb. 8 to Mar. 2, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (4 days)	207	71	11.57	136	11.38	.19
2 (11 days)	228	103	11.29	125	10.92	.37
3 (8 days)	287	196	11.21	91	10.87	.34
23 days	722	Mean difference				.30

TABLE 20

Family 58. Low line. Spine numbers during non-selection period (Mar. 2 to Mar. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (11 days)	224	125	11.38	99	10.94	.44

obtained if these two divergent branches of the low line were subjected to further selection as described above.

Thus a family containing all of the descendants of a single individual reproducing vegetatively would probably be found to consist of a large number of subfamilies, each differing slightly from the others in heritable characteristics. These divergent subfamilies would correspond in heritable characteristics to the small families derived from many "wild" individuals such as those described in part 3 of this paper (p. 105).

PERMANENT DIVERSITIES IN DIAMETER OF SHELL AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

a. Diversities in diameter of shell between the high and low lines of family 58

The question with which we are concerned here is, Do permanent diversities in measurable heritable characteristics other than spine number

appear among the descendants of a single specimen reproducing vegetatively?

It was impossible to measure all of the specimens of family 58 that were examined for spine number, but at intervals during the progress of the work measurements were made of enough of the specimens to give significant means. The data obtained are presented in tables 21 to 24 and may be summarized as follows:

TABLE 21

Family 58. Table showing distribution of diameters of shells within the high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918.) The unit of measurement is 4.3 microns.

Line	Diameters							Total number of progeny	Mean diameter
	24	25	26	27	28	29	30		
High	1	5	25	37	48	10	1	127	27.26
Low	2	10	25	65	28	6		136	26.92

Difference, .34

Measurements were made of all progeny in both high and low lines from Jan. 15 to Jan. 26, 1918, a period covering parts of the second, third and fourth non-selection periods. Table 21 gives the distribution of the diameters; the total number of progeny and the means obtained. The mean diameter of shell in the high line was greater than that of the low line. The difference of .34 unit is not great, but is as large as could be expected considering the fact that the mean difference in spine number at the same time was only .41. As was to be expected from previous tabulations a high correlation was found to exist between diameter of

TABLE 22

Family 58. Correlation table for parents and offspring with respect to diameter of the shell in both high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, .489 $\pm$.035.

		Parents						
		24	25	26	27	28	29	30
Progeny	24		2	1				3
	25		1	8	3	2		14
	26	1	5	10	24	4		44
	27		4	13	45	23	3	88
	28		1	5	17	31	8	63
	29				1	3	1	5
	30							
		1	13	37	90	63	12	217

shell and spine number (table 22). We may conclude, therefore, that permanent diversities in the diameter of the shell, as well as in spine number, have appeared among the descendants of specimen 58 during vegetative reproduction. It is remarkable how constant these characteristics are among the progeny of a single specimen, but this very constancy emphasizes the importance of the differences observed.

b. *Diversities in diameter of shell between the high and low branches of the low line of family 58*

The next question is, Is there a difference in the diameter of the shell in the high and low branches of the low line of family 58 corresponding to the difference in spine number? Measurements were made of all progeny in these two branches from Feb. 14 to Feb. 23 and from Mar. 7 to Mar. 13, 1918. The distribution of diameters of shells, the total number of progeny, and the means for both these periods and for the two periods combined are tabulated in table 23. This table shows that

TABLE 23

Family 58. Low Line. Table showing the distribution of diameters of shells within the high and low branches of the low line for two periods (Feb. 14 to Feb. 23, and Mar. 7 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Branch	Date	Diameters						Total number of progeny	Mean diameter
		25	26	27	28	29	30		
High	Feb. 14-23	4	12	42	25	8		91	27.23
Low	Feb. 14-23	1	27	56	34	6		124	27.13
High	Mar. 7-13	2	18	38	29	5	1	93	27.21
Low	Mar. 7-13	8	28	20	15	5		76	26.68
High	Feb. 14-23 Mar. 7-13	6	30	80	54	13	1	184	27.22
Low	Feb. 14-23 Mar. 7-13	9	55	76	49	11		200	26.96

Difference .26

during the period from Feb. 14 to Feb. 23, the high branch had a mean shell diameter .10 units greater than that of the low branch; that during the period from Mar. 7 to Mar. 13 this difference was .53 units; and that when the two periods are combined the difference is .26 units. At the same time the mean difference in spine number was .40.

These data prove that there is a difference between the mean diameter of the shells in the high and low branches of the low line of family 58 corresponding to the difference between them in mean spine number. The positive correlation between these characters within this family is similar to that found in a "wild" population (compare tables 24 and 1).

TABLE 24

Family 58. Correlation table for spine number and diameter of the shell in both high and low lines during the non-selection period (Jan. 13 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, $.255 \pm .042$.

		Diameter							
		24	25	26	27	28	29	30	
Spine number	9			2	2				4
	10	I	2	13	20	14			50
	11		7	16	36	24	5		88
	12		4	5	27	22	4		62
	13			I	3	7	3	I	15
	14				2	I			3
		I	13	37	90	68	12	I	222

DIVERSITIES IN DIAMETER OF SHELL AND IN SPINE NUMBER DUE TO SUDDEN LARGE VARIATIONS ("MUTATIONS")

As stated above, the constancy in both diameter of shell and spine number during the vegetative reproduction of *Arcella dentata* is remarkable. The difference in diameter of shell between parent and offspring is seldom greater than 2 units (each unit being 4.3 microns) and that in spine number usually not more than 1 or 2. In four cases there appeared in the cultures under observation, differences in diameter of shell between parent and offspring that were so great as to be conspicuous. In all of these cases the parents belonged to the low line (**E**) and the offspring were smaller than their parents. Three of the branches established by these small offspring can be disposed of very briefly; the other two deserve more extended treatment.

Branch **EF**

Specimen **EF** was the ninth offspring of a parent (58.I.I.I.4.2.I.I.I.I.I.I.I.I) that had nine spines and measured 25 units in diameter. **EF** also had nine spines but measured only 22 units in diameter. At the time **EF** appeared (December 22) the mean spine number of the low line (**E**) was 10.48 and the mean diameter 26.81 units. Part of the descendants of **EF** are shown in pedigree form in figure 18. The diameter of shell and spine number are small in the first generation, but increase to

FIGURE 18.—Part of the pedigree of branch EF. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

the mean of the line in the third generation. The small diameter of **EF** was therefore only temporary and probably due to some environmental influence, and the small diameters of the first and second generations indicate that when the decrease in size is not due to a change in the germ plasm, it requires about three generations for the descendants of a very small specimen to reach the normal size.

FIGURE 19.—Part of the pedigree of branch E421. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

Branch E₄₂₁

The progenitor of this branch, whose full label is 58.I.I.I.4.2.I.I.I.I.4.2.I., arose on December 8 from a specimen with nine spines and a diameter near the average of the parent line. Figure 19 gives part of the pedigree of this branch and shows that although small specimens still appeared in the fourth generation, there was a gradual increase in diameter and spine number that would probably have resulted in the attainment of a mean spine number of about 10.69 and a mean diameter of shell of about 26.81 which were those of the line **E** (on December 27, 1917), from which this branch was derived. It is, of course, possible that some of the sub-branches may have remained small, but lack of time necessitated the elimination of the entire branch.

Branch EM

Specimen **EM** was also a member of branch **E** of the low line. Its entire label is 58.I.I.I.4.2.I.I.I.I.I.I.I.I.I.3. It was regular in form, had 8 spines, and was only 18 units in diameter (figure 22, **B**); whereas the mean spine number of the low line at the time **EM** appeared (December 13) was 10.69 and the diameter (on December 27) was 26.81

TABLE 25

Family 58. Branch EM. Table showing the number of progeny and their mean diameter for ten generations (Dec. 13, 1917, to Jan. 19, 1918). The unit of measurement is 4.3 microns.

Number of generation	Number of progeny	Mean diameter
1	10	23.40
2	32	25.34
3	54	26.01
4	52	26.34
5	47	26.31
6	41	26.41
7	27	27.11
8	18	27.00
9	24	26.62
10	98	26.51
Total	403	

TABLE 26

Family 58. Branch EM. Correlation table for spine number and diameter of shell of 97 specimens. The unit of measurement is 4.3 microns. Correlation $.368 \pm .059$.

	Spine number					
	8	9	10	11	12	
Diameter						
23		2	1			3
24		1	3	2		6
25	1	1	14	6	1	23
26	1	2	23	19	6	51
27			3	8	1	12
28				1	1	2
	2	6	44	36	9	97

units. The parent of **EM** had 10 spines and was 27 units in diameter. This parent gave rise to offspring normal in diameter and spine number both before and after **EM** appeared. Measurements were not made of the specimens but the spine number is indicated in the pedigree shown in figure 20.

By December 29, the descendants of **EM** had increased to 58 and it became necessary to eliminate some of them. Since the diameter and spine number seemed to increase at each succeeding generation, it was decided to try to get enough specimens in each of the first ten generations to furnish significant means for each generation. As tables 25, 26 and 27 show, **EM** produced ten offspring whose mean diameter was 23.40. There was a gradual increase in the mean diameter during the first four generations (figure 21) and then fluctuations until the tenth generation (January 19, 1918) when the work was stopped. During the period when these progeny were obtained (December 13, 1917, to

FIGURE 20.—Pedigree showing the parent of specimen **EM** and five of the sisters of **EM**. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

January 19, 1918) the mean diameter of the low line **E** from which **EM** arose was 26.81 units (on December 27, 1917) and the mean spine number 10.62. These data indicate that the divergence of **EM** and the first 4 or 5 generations derived from it was only temporary and that **EM** could not be called a mutation in the sense that its germ plasm had become permanently modified. As noted above in the case of branch **EF** (page 128) when for some reason a specimen appears that is markedly different in size from its parent, this decrease in size being due to some environmental factor, it seems to require several generations before the parental condition is regained. At certain times during the cultivation of branch **EM** sudden increases and decreases in the diameter and spine numbers of all the progeny occurred similar to those noted in the other

Family 58. Branch EM. Table showing a notable increase and subsequent decrease in spine number and in diameter of the shell. The unit of measurement is 4.3 microns.

Date	Number of progeny	Mean spine number	Mean diameter
Feb. 2	9	11.22	27.55
Feb. 7	7	12.28	28.85
Feb. 9	8	10.87	27.50

branches of family 58 (see page 121). Table 27 gives the data for a few days during which a notable increase and subsequent decrease took place.

Branch ED

The work carried on with this branch was more extensive than with the other branches described above, and led to the greatest divergences that were discovered during the entire work. The specimen **ED** appeared on December 22, 1917. Its complete label is 58.1.1.1.4.2.1.1.1.1.1.1.1.8. It was the eighth offspring of a specimen that was of average size and whose other offspring were of average size. **ED** itself is very close to the mean of the race, measuring 26 units in diameter and possessing 9 spines. The line from which it arose had a mean spine number at this time of 10.48 and a mean diameter of 26.81 units. **ED**

FIGURE 22.—Members of the branch **EM**. **A**, the parent of **EM** with 10 spines and a diameter of 116 microns. **B**, **EM** with 8 spines and a diameter of 77 microns. **C**, the first offspring of **EM**, with 9 spines and a diameter of 99 microns. **D**, a typical member of the fourth generation with 10 spines and a diameter of 116 microns. $\times 207$.

produced 8 offspring with a mean diameter of 20 units and a mean spine number of 8.80. The diagram on page 133 (figure 24) gives a portion of the pedigree of this branch; figure 23 on the same page indicates graphically the principal phases of the work; and table 28 gives the mean diameters and spine numbers of the generations from 1 to 24.

All parents and offspring in this branch were kept for a period of 20

FIGURE 23.—Diagram showing data obtained during the cultivation of branch ED. The data are arranged as in figure 11.

FIGURE 24.—Part of the pedigree of branch ED beginning with specimen ED. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns. The letter E indicates that the shell was empty.

days until there were 123 in all. Then a representative lot of 22 specimens were taken and for a period of 27 days all parents were eliminated

TABLE 28

Family 58. Branch ED. Table showing the mean diameter and mean spine number for generations 1 to 24 (Dec. 22, 1917 to Feb. 22, 1918). This table includes the large specimens belonging to the subbranch EDA, but not the members of the subbranches EDB and EDC. The unit of measurement is 4.3 microns.

Number of generations	Number of specimens	Mean diameter	Mean number of spines
1	8	20.00	8.80
2	12	21.75	9.25
3	28	22.78	9.74
4	28	23.57	10.10
5	20	24.30	10.00
6	22	24.13	10.54
7	15	23.60	9.66
8	10	23.50	10.10
9	9	23.88	10.22
10	13	24.00	10.30
11	9	23.44	10.22
12	8	23.12	9.37
13	6	23.83	9.33
14	6	23.33	9.66
15	14	23.57	9.57
16	24	23.50	10.08
17	19	23.42	9.84
18	6	24.33	9.33
19	15	24.53	10.33
20	15	24.00	10.06
21	11	23.73	10.00
22	8	23.37	10.25
23	5	24.00	10.40
24	1	24.00	9.00
I-24	312	23.51	9.91

as soon as they had reproduced and the offspring were kept. Then for 11 days both parents and offspring were kept. During these three periods, totaling 58 days, records were made of 312 offspring. These had a mean diameter of 23.51 units and a mean spine number of 9.91. During the same period the low line **E** from which **ED** arose had a mean diameter of 27.05 and a mean spine number of 10.99. There is thus a difference in diameter of 3.54 units and in spine number of 1.08.

In the fifth and sixth generations of this branch there appeared in one subbranch a remarkable increase in diameter and in spine number, as shown in the diagram on p. 137 (figure 26, **EDA**). This increase may be tabulated as follows:

Generation	Spine number	Diameter
1	8	22
2	8	24
3	10	24
4	11	26
5	13	31
6	16	32

Several empty shells were thrown by members of this subbranch, but empty shells were of rather frequent occurrence in all the lines studied and in the other lines did not cause any such remarkable changes. There were only three very large specimens produced; these measured respectively 31, 31, and 32 units in diameter, and possessed 13, 14 and 16 spines respectively. No further offspring were obtained from these nor from their progenitors although every care was taken to keep them in favorable cultural conditions.

On February 20 it was decided to try selection with those members of the branch **ED** that remained alive at that time. Four specimens were selected on the basis of past performance to start a high line and 4 to start a low line. The diameters and spine numbers of these were as follows:

High line		Low line	
Diameter	Spine number	Diameter	Spine number
24	10	22	9
25	11	22	10
25	10	22	9
26	12	19	9

During a period of 9 days fifty-five offspring were produced in the high line with a mean diameter of 27.07 units and a mean spine number of 11.16, and 21 offspring in the low line with a mean diameter of 23.61 and a mean spine number of 10.10 (table 29).

Twenty-two representative specimens were then taken from the high line and all of the 21 in the low line were kept. All parents were eliminated as soon as they reproduced and the offspring retained. The number of offspring and means obtained during this period of 13 days are given in table 29.

Four specimens were then selected from the high line for the purpose

TABLE 29

Family 58. Branch ED. Table showing mean diameter and mean spine number during one period (Feb. 20 to Feb. 28, 1918) and a subsequent non-selection period (Mar. 1 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Period	Number of progeny	High branch			Low branch			Mean difference	
		Number of progeny	Mean spine number	Mean diameter	Number of progeny	Mean spine number	Mean diameter	Spine number	Diameter
Selection (9 days)	76	55	11.16	27.07	21	10.10	23.61	1.06	3.64
Non-selection (13 days)	224	109	12.06	28.09	115	9.94	23.66	2.12	4.43

300

of determining how large a line could be obtained. These four gave rise to 11 offspring and then stopped reproducing (figure 26, EDB). No more offspring appeared, although the specimens were very carefully looked after. These 11 offspring had a mean diameter of 35.54 units and a mean spine number of 17.54. The largest specimen measured 40 units in diameter and possessed 20 spines. This is the largest specimen discovered during the entire course of the work. Arcellas with from 13 to 15 spines and with a diameter of from 34 to 36 units were taken from the pond and many of them reared in the laboratory in other lines, and

FIGURE 25.—Members of the branch ED. A, the progenitor of the branch ED with 9 spines and a diameter of 112 microns. B, the first offspring of ED, with 9 spines and a diameter of 99 microns. C, the second offspring of ED, with indistinct spines and a diameter of 82 microns. D, the third offspring of ED, with 8 spines and a diameter of 95 microns. E, the largest specimen from the subbranch EDB, with 20 spines and a diameter of 172 microns. $\times 207$.

FIGURE 26.—Diagram showing the magnitude of the diversities in spine number that appeared during the vegetative reproduction of family 58. The distances between the horizontal lines indicate the differences between the mean spine numbers of the lines studied. A mean difference of one spine is equal to a vertical distance of 37 mm in the diagram. The diagram shows that before selection was begun, the mean spine number of the members of the family was 10.87. A high line (H) and a low line (L) were obtained during six selection periods with a final difference of 11.48 — 10.32, or 1.16. The high and low lines remained different during four succeeding non-selection periods, the final difference being 11.69 — 11.26, or .43. Selection was then started in the high line and was continued for one period resulting in a difference of only .01 between the high branch AG and the low branch AH. Selection was also begun in the low line at the same time and was carried on for three periods, EG and EH, a final difference of 11.21 — 10.87, or .34, being obtained. This was followed by a single non-selection period which ended with a difference of 11.38 — 10.94, or .44.

During the third selection period a very small individual, EM, appeared in branch E, but this proved to be only a temporary divergence since its descendants by the end of three periods had attained a mean spine number (10.65) at least equal to that of the parent branch E.

During the fifth selection period an individual, named ED, appeared in branch E and produced descendants with a much lower spine number than the parent branch. The main branch ED remained at a lower level than the parent branch E, but three branches, EDA, EDB and EDC originated from it which reached a higher mean spine number than any other lines studied, namely, 13.15 in branch EDA, 17.54 in branch EDB, and 12.80 in branch EDC. More detailed data will be found in diagram 11 on page 110, and several other branches that were studied will be found described in the text, having been omitted from the diagram for the sake of simplicity.

even specimens with 16 or 17 spines and a diameter of 37 units were recorded, but none were found that approached this in number of spines or in diameter except in this particular branch of the line **ED**.

At the same time that the high line in this branch **ED** was being studied, an attempt was made to obtain a further divergence between the specimens in the low line. Two specimens were selected for the high line and two for the low line as follows:

High line		Low line	
Spine number	Diameter	Spine number	Diameter
10	27	10	24
12	27	9	22

Only five offspring were produced by the two specimens selected for the high line (figure 26, **EDC**). These had a mean diameter of 28.20 units and a mean spine number of 12.80. No further offspring appeared and all of the specimens died.

In the low line after the 2 selected specimens had increased to 22, parents were eliminated and offspring kept whenever reproduction occurred. This procedure was maintained for 42 days (from March 14 to April 24, 1918). Table 30 shows the correlation between diameters and spine

TABLE 30

Family 53. Branch ED. Correlation table for spine number and diameter of shell in low subbranch of the low branch (March 14 to April 24, 1918). The unit of measurement is 4.3 microns. Correlation, $.531 \pm .041$.

	Spine number						
	7	8	9	10	11	12	
16	1						1
17							0
18							0
19							0
20			2				2
21			2				2
22	1	1	11	5	1		19
23		1	18	31	9		59
24		1	8	21	13	3	46
25			1		7		8
26					2		2
	1	3	42	57	32	3	139

numbers during this period. The mean spine number of the 139 progeny recorded is 9.88 and the mean diameter of the same progeny is 23.23

(see figure 23). All progeny were below the mean diameter of the line from which their original progenitor was derived and the mean diameter of this branch is nearly the same as the mean of the first large group of 312 reared before selection was begun (figure 23).

It seems clear, therefore, that the line **ED** had become permanently different from the original line **E** from which it arose both with respect to diameter and to spine number. It seems strange that this small derivative of the original line **E** should give rise to three branches **EDA**, **EDB** and **EDC** which are larger than any other branches in the entire family 58 (figure 26). Another phenomenon that needs to be accounted for is the dying out of all of the members of these large branches. The probable reason for this will be pointed out in a later paper, where the relations between nucleus and cytoplasm are described.

Some time after branch **ED** appeared it was discovered in another line that specimens with a single nucleus could be obtained by removing one of the two normally present, and that these uninucleate specimens would produce uninucleate progeny. These uninucleate progeny in every case had shells that were smaller than those of their modified parents as well as fewer spines. All uninucleate progeny that appeared later likewise possessed smaller shells. This discovery led to an examination of the nuclear condition of the members of branch **ED** since the small size of these specimens might be due to the presence of only one nucleus instead of the normal number, two. It was impossible to determine with certainty the nuclear condition of all of the progeny of **ED** because of their small size, the color of the shell, and the large amount of food material often contained in them, but in a large number of cases two nuclei were plainly visible even in some of the smallest specimens measuring 22 units in diameter or less, and only in three or four specimens was it possible to conclude that only one nucleus was present. If the few cases of this kind were thrown out of the foregoing calculations, they would make practically no difference in the results.

EMPTY SHELLS

At irregular intervals during the course of this investigation new shells were formed which separated from the parent organism without being provided with any protoplasm. These empty shells appeared in all the lines under cultivation and no factors were discovered that would account for their production. In family 58 a total of 59 empty shells was recorded or about one percent of the specimens studied in this entire family. Since the empty shells were almost always smaller than their

TABLE 31

Family 58. Table showing the relations between empty shells and their parents and older and younger sisters, with respect to spine number and diameter in ten cases. The unit of measurement is 4.3 microns.

Parent		Empty shell			Older sister		Younger sister	
Spine number	Diameter	Progeny number	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter
11	27	4	8	22	10	25	9	26
12	29	2	9	20	12	28	11	26
10	24	2	8	19	11	24	12	27
9	20	2	7	18	10	22	9	22
9	24	2	8	19	10	25	9	19
11	26	2	10	20	12	27	12	26
11	26	8	8	22	13	27	11	27
8	23	2	9	21	10	24	9	23
8	19	2	7	19	9	20	9	22
9	22	3	7	20	9	22	7	21

parents and possessed a lesser number of spines, they were not included in the data presented in the preceding pages.

Table 31 gives the data with regard to ten representative empty shells. In a few cases the number of spines possessed by the empty shell could not be determined; in a number of cases the diameters of the empty shells, parents, and sisters were not obtained; and in 35 instances the empty shell represented the first offspring and hence had no older sister, and some of the parents were eliminated before a younger sister was produced.

The data obtained show that of the 59 empty shells in family 58,

35 were the first offspring of their parents;

12 were the second;

4 were the third;

3 were the fourth;

3 were the fifth; and

2 were the eighth.

The comparatively large number belonging to the first and second generations is no doubt due to the fact that most of the specimens in the family were eliminated after producing one or 2 offspring. The empty shells possessed fewer spines than their parents in 45 cases, a greater number in 5 cases, and an equal number in 2 cases. They were smaller than their parents in 38 cases; larger in 2 cases, and equal in size in 2 cases.

The relations between the empty shells and their older and younger sisters, i.e., the progeny of the same parent just preceding or succeeding them, were determined with the following results. The spine number of the older sister was greater than that of the empty shell in 18 cases, less in 1 case, and equal to it in 1 case. The spine number of the younger sister was greater than that of the empty shell in 47 cases, less in 2 cases, and equal to it in 5 cases. The spine number of the older sister was greater than that of the younger sister in 6 cases, less in 5 cases, and equal to it in 2 cases. The spine number of the parent was greater than that of the younger sister in 16 cases, less in 21 cases, and equal to it in 5 cases. It seems evident from these results that the production of empty shells has no appreciable influence upon the spine number of later offspring (younger sisters) from the same parent.

Similar relations have been found with regard to the size of the empty shells, their parents, and older and younger sisters. These relations are as follows:

Empty shells were smaller than the parents in 38 cases;

larger in 2 cases;

equal in 3 cases.

Empty shells were smaller than the older sisters in 16 cases;

equal in 1 case.

Empty shells were smaller than the younger sisters in 36 cases;

equal in 2 cases.

Younger sisters were larger than the older sisters in 5 cases;

smaller in 6 cases;

equal in 2 cases.

Younger sisters were larger than parents in 19 cases;

smaller in 12 cases;

equal in 5 cases.

These data show that the empty shells are almost always smaller than their parents, and smaller than their older and younger sisters. The influence of the production of the empty shell upon the succeeding offspring (younger sister) from any given parent is indicated in the last set of data presented above. The next offspring produced after an empty shell is formed is in more than half of the cases larger than the parent. Why this should be so is unknown, but the empty shells are doubtless empty because of a disturbance at the time of reproduction and probably there was not sufficient protoplasm for the production of a normal individual. If this were the case there would be a superabundance of protoplasm ready by the time of the next fission and the next offspring

(younger sister) would tend to be larger than its parent. However, there is no appreciable difference in size between the offspring preceding and the one succeeding the production of the empty shells, so until more extensive data are available the causes and effects of empty shell formation will have to remain unexplained.

It is worthy of note, nevertheless, that two of the sudden increases in diameter and spine number in branch **ED** (see figure 26) were both preceded by the formation of empty shells. In a number of other cases, on the other hand, sudden increases in size and spine number occurred in all the lines under cultivation at the same time without the intervention of empty shell production.

In a number of experiments on *Arcellas* taken from family 58 or directly from the pond, it was found that specimens when deprived of one of their two nuclei always formed an empty shell just before the binucleate condition was regained. Apparently nothing of this kind happened in the 59 cases described above, since in many specimens the nuclear condition was examined and two nuclei were always present (HEGNER 1919).

Conclusion

We may therefore ignore the empty shells in our studies of diversities in these organisms, at least until we know more about the conditions that cause their formation.

DISCUSSION

A number of questions have suggested themselves during the course of these studies which may profitably be grouped together in this discussion.

1. How does selection operate when practiced on *Arcella* during vegetative reproduction?

This question is of considerable importance since the significance of the results recorded in this contribution can not be realized until the fundamental difference between selection among sexually and asexually reproducing organisms is clearly understood. If it had been possible to take care of all of the descendants resulting from the vegetative reproduction of the single individual (No. 58) with which these lines were started, and if the pedigree of all these descendants were constructed according to the scheme indicated on page 110, the same lines and branches would appear in this pedigree that have been described in the foregoing pages, but besides this hundreds of other branches would also appear,

FIGURE 27.—Diagram showing the various heritably diverse branches with respect to spine number and diameter that may arise from a single specimen of *Arcella dentata* during vegetative reproduction. **OP** = original progenitor. A high line **A** and a low line **B** are isolated by selection. The means of these lines then regress slightly toward the mean of the entire family, but soon become almost stationary although branches from both lines cross each other. For further explanation, see page 145.

such as are indicated in figure 27, branches that were eliminated during the experiments because of lack of time and strength to keep them going. In other words, there has been no selection of existing heritable factors that are contributed to the progeny by two different parents, as is usually the case when selection is practiced among sexually reproducing organisms; but specimens have been picked out from a pedigree resulting from the successive divisions of a single germ plasm.

2. Are shell characteristics influenced by the environment?

There is a peculiarity exhibited by many of the correlation tables that have been made up that needs consideration. Offspring of parents with a spine number near the lower limit, e.g., those of parents with 8 or 9 spines, possess an exceptionally high mean spine number.

Two sets of factors must be considered in interpreting such tables, (1) hereditary factors and (2) environmental factors. It seems perfectly clear that the influence of heredity is very strong. As regards the

environment, although this was kept as uniform as possible at all times, still small solid objects such as minute grains of sand might modify the shell of the offspring while it is still in a plastic condition, so as to prevent the development of the complete (hereditary) spine number. This would account for *some* of the offspring with 7, 8 and 9 spines that were produced by parents with 11 and 12 spines. The hereditary factor would tend to express itself again in the next generation, hence, e.g., offspring with 8 spines would produce offspring with 10, 11, 12 or 13, as shown in the tables. This may account for the exceptionally high mean spine number of the progeny of parents with 8 spines as shown in table 5. Many of the low-spined specimens here recorded were not regular, but, as indicated in figure 12, **C**, large spaces sometimes occurred which might be due to the suppression of one or more spines by some obstruction in the environment.

Mechanical environmental factors might thus cause a decrease in spine number, but probably could not produce an increase; thus the mean spine number of the offspring of high-spined parents would tend to be lower than their hereditary constitution. Other environmental factors may affect the organism at the time of reproduction or during the intervals between fissions. In the latter case the results of their influence would not be visible until reproduction occurs, and then only in the offspring, since the shell of the parent is not modified after it is once formed.

In this connection we may also refer to the increases and decreases in spine number that have several times been found to occur simultaneously in all of the lines under cultivation. Examples of this are cited in both the high and low lines of family 58 (page 121) and in branch **EM** (page 131), and data are presented in tables 17 and 27. The most plausible suggestion to account for this is that some cultural condition favorable for the growth of the organisms caused an increase in size, and a subsequent less favorable condition resulted in a reversion to the previous state which we have called "normal." This increase in size would be accompanied by an increase in the spine number, since these two characters are closely correlated.

3. Why was there a decrease in the amount of diversity between the high and low lines of family 58 after selection was discontinued?

The lesser difference between the high and low lines at the end of the non-selection periods than at the beginning is probably due to the appearance in the high line of heritable variations toward a lower spine number, and in the low line of similar variations toward a higher spine num-

ber. Specimens exhibiting such variations would have been removed during the selection periods and hence would not have been allowed to produce offspring which would lower or raise the means in the two lines.

In other words, the differences between the means of the two lines during the selection periods appear greater than they really were, and when selection was stopped, the true condition was revealed. From this time on there should be no great fluctuations in the magnitude of the difference between the means except those due to environmental influences. This proved to be true, as the data show clearly.

Figure 27 was made on the basis of the data presented in this paper to illustrate what probably happens during the vegetative reproduction of *Arcella dentata*. Part of the hereditarily diverse branches that result during the vegetative reproduction of a single specimen are here shown. Suppose for purposes of illustration that we have by selection picked out of the entire pedigree the high line **A** and the low line **B**. If we then discontinued selection we would obtain a pedigree in which lines **A** and **B** would give rise to the branches **AA**, **AB** and **BA**, **BB**, and from these in turn would be derived various other branches, a few of which are indicated in the diagram. Some of these branches would have a mean similar to **A** and **B**, but other branches would have a mean either above or below that of the line from which they sprang. Finally an upper limit, represented in the diagram by branch **AAAA**, would be reached beyond which an increase in size and spine number is not possible, just as there is a well known limit to the size of all species of organisms. And similarly a lower limit. **BBBB**, would also be reached. Some of the branches of the high line **A**, such as **ABBB**, would coincide with or even possess a lower mean size and spine number than certain branches of the low line **B**, such as **BAAB**. And certain branches of the low line **B**, such as **BAAA**, would become higher than some of the branches of the high line **A**, such as **AACB**. The mean size and spine number of the entire group of specimens obtained after selection ceased (**S** in figure 27) would at first decrease slightly in line **A** and increase slightly in line **B**, but thereafter would fluctuate very little and the two lines **A** and **B** would remain distinct indefinitely.

The fact that there appears to be both an upper and a lower limit to the size and spine number in these organisms may account for the slight difference of .07 during the second non-selection period (see figures 11 and 26). When the high line reached a mean spine number of 11.90 during the first non-selection period, its upper limit for this character had about been reached. After this upper limit had been reached by the high

line, the low line continued to increase in spine number until it almost attained this upper limit, having a mean spine number of 11.84 in the second non-selection period. Then when the environment changed and a decline in spine number set in, the mean of the low line decreased more than that of the high line and a difference of .41 resulted during the third non-selection period. A simultaneous increase in spine number again appeared during the fourth non-selection period but at this time there was no near approach to the upper limit and the increase in the high line (.27) was greater than that in the low line (.25).

5. Are diversities due to large or small heritable variations?

The studies of family 58 suggest that the diversities obtained were due to barely distinguishable heritable variations and that an actual shifting of the mean and the mode has occurred in this way. The establishment of branch **ED**, however, as a permanently small line, proves that hereditarily diverse lines may be produced by the appearance of a few extreme specimens. The fact should not be overlooked that when an extreme specimen arises, such as **EM** (page 129), whose diversity is not hereditary, it requires several generations before the mean condition of the line is regained. It may therefore also be true that a large change in the hereditary constitution of a specimen would not appear in its entirety in the first generation, although the f_1 progeny would exhibit it in part, but would become fully manifest only in about the third or fourth generations. While it does not seem probable that the high and low lines of family 58 have originated in this way, still it is a possibility that must be kept in mind.

It also seems probable that mutations may have occurred more often than actually observed, since, because they were not visible in the first generation, they might have been discarded. Especially is this true of mutations toward a lower spine number in the high line and a higher spine number in the low line. This may account for the fact that the supposed mutations discovered in the latter were all smaller than the mean of the line.

6. How do diversities in heritable characteristics originate during vegetative reproduction in *Arcella dentata*, i.e., what is the method of evolution in these organisms?

The germ plasm of organisms that are producing vegetatively is commonly supposed to divide into two qualitatively equal parts at each division and the descendants therefore should all be alike. The fact that branches of such a family as No. 58 are permanently diverse, proves that changes have occurred in the *constitution* of the germ plasm during vegetative reproduction; that is, evolution has taken place. These changes in

the constitution of the germ plasm may be due to two causes, (1) unequal distributions of qualitatively different germinal materials (factors or parts of factors?) during fission, or (2) actual changes (chemical?) in the germ plasm arising spontaneously or initiated by the internal or external environment.

The presence of a conspicuous network of chromidia in *Arcella*, which has been identified as idioplasm, may lead during fission to differences in parent and offspring in chromatin content which would account for the heritable diversities discovered. The removal of part of this chromidial network, however, as will be shown in another paper, has no effect upon the heritable characteristics of the line. It may also be that nuclear reorganization processes occur during vegetative reproduction, such as take place in *Paramecium*, but nothing of the kind has been observed in the thousands of specimens examined, although the nuclei may easily be seen in the living animal. This problem can not profitably be discussed further at this time, since, although more is known about the nuclear phenomena and reproductive processes in *Arcella* than in any other rhizopod, still many of the investigations need to be checked up and there is much still to be learned. *Arcella*, however, offers many advantages for the study of the problem of the method of evolution and further investigations along this line are now in progress.

7. How do the results obtained from these studies of *Arcella dentata* compare with those previously reported by other investigators?

Most of the investigators who have attempted to change the genotype by selection in Protozoa that are reproducing vegetatively, have failed.² MIDDLETON (1915), however, succeeded in obtaining from a single progenitor two lines of *Stylonychia* that differed in fission rate, and JENNINGS (1916) demonstrated the fact that vegetative reproduction in *Diffugia corona* is accompanied by minute heritable variations in a number of measurable characters resulting in branches that differ markedly in their genotypic condition. The investigations reported in this paper show that *Arcella dentata* resembles *Diffugia corona* in this respect and confirm many of JENNINGS's discoveries. Since these two organisms resemble each other very closely, it may be worth while to compare briefly the results of JENNINGS's investigations on *Diffugia* with those reported herein on *Arcella*.

Among the characters of *Diffugia corona* studied by JENNINGS are spine number and diameter. The spines of *Diffugia corona* vary from

² For the literature on this subject see JOHANSEN (1913), MIDDLETON (1915), JENNINGS (1916), ACKERT (1916), ROOT (1918).

0 to 14, those of *Arcella dentata* from 7 to 20. In both organisms, (1) families resulting from the vegetative reproduction of single "wild" specimens were obtained which were hereditarily diverse with regard to spine number; (2) families diverse in shell diameter were discovered; (3) deviations of the parents from the mean spine number and diameter are in part inherited by the offspring; (4) diameter and spine number are closely correlated, and the greater the diameter the more numerous are the spines; and (5) the long-continued selection of the progeny of a single specimen that shows deviations in these characters results in the isolation of lines that are heritably diverse. Mutations occur in both *Diffugia* and *Arcella*, but diversities in lines derived from a single specimen seem to be due rather to the accumulation of many small heritable variations than to large ones.

SUMMARY

1. The main problem attacked in this investigation is, Can heritably diverse lines be recognized among the descendants of a single specimen of *Arcella dentata* produced by vegetative reproduction?

2. *Arcella dentata* was chosen as material because it possesses several definite measurable characters, especially the number of spines and diameter of the shell, which are fully determined at the time of reproduction and are not thereafter affected by growth changes, or by environmental factors (figure 1).

3. "Wild" specimens vary in spine number from 7 to 17 and in diameter from 73 microns to 150 microns; these characters are correlated, and on the average the greater the diameter, the more numerous are the spines. (Tables 1 and 2.)

4. Variations in spine number occur among the descendants of a single specimen produced by vegetative reproduction, and these variations are in part inherited.

5. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number and diameter. A "wild" population therefore consists of a large number of hereditarily diverse families that may be isolated in the laboratory. (Tables 3, 4 and 5.)

6. A single large family (No. 58), containing 5557 specimens and representing 69 generations, was obtained from a single specimen by vegetative reproduction. Selection within this family led to the isolation of hereditarily diverse branches as follows (figures 11 and 26).

(a) Using mostly past performance as a basis for selection, two lines

were obtained during a period of 64 days and involving the study of 1192 specimens, representing 22 generations. The difference in spine number between these two branches at the end of this period was 1.16 and the mean difference for the entire period was .55. A difference in spine number was maintained during a succeeding non-selection period of 35 days; 1325 specimens and 18 generations were studied during this period. The difference in spine number between the high and low lines persisted throughout the non-selection period, but decreased to an average mean of .46. This was to be expected since hereditary diversities are constantly appearing both toward an increase and a decrease in spine number (see page 145). (Tables 6 and 14.)

(b) The low line thus obtained was subjected to a period of selection of 23 days during which 722 specimens were obtained, representing 15 generations. Two hereditarily diverse branches of this low line appeared with a mean difference in spine number of .30 which increased during a short succeeding non-selection period. (Table 19.)

(c) Measurements of the diameters of the low and high lines mentioned in (a) show that these lines also were diverse with respect to the diameter of the shell and that spine number and diameter are correlated throughout the entire family. The mean difference in diameter between the high and low lines was .34 units.³ (Tables 21 and 22.)

(d) Similarly there was a mean difference of .26 units in diameter between the high and low branches of the low lines. (Table 23.)

(e) Four branches of family 58 were studied whose progenitors were markedly smaller and possessed a lesser number of spines than the other specimens in the low line in which they appeared. Three of these branches reverted to the mean condition of the low line within a few generations, but one of them, **ED**, proved to be permanently diverse in both spine number and diameter. It differed from the mean of the low line by 1.08 in spine number and by 3.54 units in diameter. (Figure 23.)

(f) From this branch **ED** there appeared at different times three distinct branches (**EDA**, **EDB**, and **EDC**) containing specimens larger and with more numerous spines than those encountered in any other part of the entire family 58. (Compare figures 13 and 25.)

7. Empty shells are often produced by apparently normal specimens. They are almost always smaller than their parent, and smaller than their older and younger sisters. Their appearance seemed to have no influence upon the heritable diversities studied. (Table 31.)

³ The unit of measurement is 4.3 microns.

8. A large family of *Arcella dentata*, therefore, derived from a single specimen by vegetative reproduction, consists of a number of branches that are hereditarily diverse with respect to diameter and spine number. These diverse branches resemble the hereditarily diverse families that were obtained by vegetative reproduction from different "wild" specimens.

9. The formation of such hereditarily diverse branches appears to be a true case of evolution that has been observed in the laboratory and that occurs in a similar way in nature.

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