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TITLE

Colony Development of a Polymorphic Hydroid as a
Problem in Pattern Formation¹*

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SYNOPSIS

This is an attempt to simulate on an electronic computer the development of the marine hydroid Podocoryne carnea as a colony is formed by a single hydranth placed on a microscope slide. The computer generates a relatively complex pattern as a mathematical model of the growing system by iteration of a set of simple rules of growth. The model consists of a connected network of points in a two-dimensional net. Its growth can be followed generation by generation and selected parameters of the generated pattern compared to those of real colonies. The ability of simple recursive rules to generate complex patterns suggests the possibility that genetic instructions of developing systems may, in part, be of a similar nature.

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INTRODUCTION

Podocoryne carnea is a polymorphic, colonial, marine hydroid, normally appearing on a snail shell inhabited by a hermit crab. The various hydroid persons constituting a colony include the nutritive, the sexual, and the spiral zooids (Fig. 1). These are connected at their bases by a branching stolon network. Podocoryne bears free-swimming medusae and is dioecious.

A single hydranth plucked from the shell-borne colony will attach to a microscope slide in standing sea water, within a few days. Stolons grow out from the initiating hydranth; they branch and anastomose. On these stolons new hydranths appear. Colonies initiated in this manner ultimately bear nutritive and sexual zooids (Pl. 1, fig. 1). Spiralzooids never form on them.

Colonies established in this manner can be maintained under defined conditions (Braverman, 1962) and their daily changes in form recorded by photograph or camera lucida drawings (Pl. 1, fig. 2). Although there appears to be no recognizable pattern to colony morphology, there is an underlying statistical regularity. The growth of the colony (Fig. 2) proceeds at a constant rate, and the proportion of hydranth number to stolon length is regulated at a constant proportion (Braverman, 1963). It further appears that the distance between hydranths of young colonies tends toward a norm.

Measurements of the interhydranth distance in these same colonies when they are older indicate that with increasing age (perhaps reflecting increasing stolon connectivity) the average interhydranth distance decreases (Fig. 3).

The constant rate of growth continues until the time when sexual zooids appear in the colony. At that time the rate of nutritive zooid formation is depressed. The sexual zooids' involvement in growth control, or the coincidental effect of some other controlling factor on both sexual and nutritive zooids, is indicated by long-term qualitative analysis (Fig. 4).

In one month old colonies of clone X grown at 23° C, hydranths fall into three size groups. There are newly formed hydranths of graded size, whose size indicates their age. There are older hydranths that are larger than the growing hydranths, but all the same size, and there is the primary hydranth, the hydranth used to initiate the colony, which is the largest of all. Apparently the growth of hydranths in these asexual colonies is limited, as none grows so big as the initiating hydranth.

By two months sexual zooids have appeared at the center of the colony, and hydranths in the sexual area and that immediately surrounding it have grown to the size of the initiating hydranth. The other two areas of hydranth size: the graded, growing hydranths, and those of the same size but not of maximum size, still appear in the colony, but are now in more peripheral areas. At one month only a small proportion

of the most peripheral stolon area is devoid of hydranths; at two months, however, there is a large peripheral stolon area with few or no hydranths growing on it.

During the third month, each area extends peripherally; new hydranths grow on the formerly bare stolons; hydranths that were of graded sizes at three months grow to the limited maximum size, and those that had been limited maximum size before attain the larger size, as the sexual area extends peripherally.

Finally, colony growth is halted by the limits of the slide, and when the expanding sexual area reaches the limits of the colony, the colony undergoes a major change, resulting in a reduction in both nutritive and generative zooid density and an increase in the proportion of sexual zooids.

Between the fourth and fifth month glossy areas of 0.5 to 2.0 mm. in diameter appear on the colony in what seems to be a random pattern--although their distances from each other appear to be roughly the same. Apparently the radial orientation of the colony no longer exists. These glossy areas become bright yellow in time. They never acquire the configuration of the spines that exist on shell-borne colonies, nevertheless it is conceivable that they might bear some relation to that structure.

The existence of statistical regularity in the colony, the proportional growth of stolons and hydranths, and the regularity of interhydranth distances, suggest that the

apparently irregular colony growth pattern is an expression of underlying rules of a non-deterministic nature. That these rules are general rather than specific is indicated by the variance in pattern, but similarity of statistics, evidenced by sibling colonies (that is, by colonies initiated with nutritive hydranths removed from the same portion of the same parent colony). It is highly unlikely that each direction, each stolon branch point, each hydranth location is specified by the genetic information of the animal.

It is more likely that genetic information in this animal consists of a series of rules of general application. For example hydranths might be inserted at any point at which the concentration of a hydranth inhibitor falls below a threshold value; stolon growth might be a function of the amount of food reaching a stolon tip through the stolon circulation, which might in turn be dependent upon the distance of that point from the food sources--nutritive hydranths; a new stolon growth point might form at any point at which an imbalance in the ratio of some stolon stuff and some hydranth stuff exists. A single asymmetry, and asymmetry could be introduced through any of these hypothetical rules, would result in a colony of no apparent pattern.

If it were true that the apparent irregularity of the Podocoryne colony reflected the interaction of a series of probabilistic rules, then it should be possible to translate any proposed mechanism for colony regulation into the form

of a generating rule, apply the rule, and see if the distribution of colony entities in the generated colony resembles that of the real colony. Affirmation would specify that the mechanism could give the observed colony shape or statistic, not that it is, in fact, the mechanism employed by the animal. Negation would rule the mechanism out.

Since the stolons of a Podocoryne colony are limited to the plane of the microscope slide substrate, and adherent to it, a one-to-one mapping of the developing colony in two dimensions is possible. Crosses and noughts can be used to represent sexual and nutritive zooids, respectively, and straight lines to represent interconnecting stolons. A colony can thus be represented as a pattern of lines, crosses and noughts on a sheet of paper.

The mathematician, S. Ulam, has demonstrated that simple rules, applied recursively, can generate complex patterns (Ulam, 1962). He gives as an example the following:

"We start in the first generation with a finite number of squares and define now a rule of growth as follows:
Given a number of squares in the n th generation,
the number of squares of the $(n+1)$ th generation will
be all those which are adjacent to the existing one
but with the following proviso: the squares
which are adjacent to more than one square of the
 n th generation will not be taken. For example starting
with one square in the first generation one obtains the
following configuration after five generations.

"It is obvious that with this rule of growth the figure will continue increasing indefinitely. It will have the original symmetry of the initial configuration (one square) and on the four perpendicular axes all the squares will be present--these are the stems from which side branches of various lengths will grow."

Even in this trivial example it is clear that what happens in each generation is a function not only of the rules, but of the environment also; and what the environment consists of in any generation is a function of previous applications of the rule, so that generation four can add a total of twelve squares while generation five can add only four.

The pattern carried out to twenty-three generations looks like this:

Although certain regularities are observable, it would be extremely difficult to derive the generating rules from the pattern, unless they were already known.

It may be worthwhile to note that were one required to send a telegram describing this pattern from, say, New York to London, it could be done in one of two ways. One way would be to describe a matrix and read off the co-ordinates of each occupied square. The other way would be to state the generating rules and then specify the number of times they are to be applied. For patterns of more than eight generations (assuming one pair of co-ordinates be counted as a word) the second method, rule specification, would be cheaper, and less prone

to error, in that a mistake in a single letter rarely renders a word unintelligible.

Ulam's provocative demonstration suggests a model for generating the patterns of a hydroid colony with simple rules. It requires that the direction of stolon growth be limited to the x and y axis of a grid of rectangular co-ordinates, and that some proviso be incorporated to translate the continuous growth of the real colony into discontinuous growth.

The parameters of colony growth that have to be defined are the following:

1. The growth of individual stolons.
2. The points and times of branch points at which the growth of a new stolon is initiated.
3. The appearance of new hydranths, nutritive or generative.
4. The size to which hydranths grow. (The assumption of size equality has been made to simplify the initial problem.)

In order to represent continuous growth in a discontinuous model, the expedient of providing for probabilistic growth of a constant amount can be adopted. Thus, instead of growing continuously at a rate, say, of 0.75 stolon units per day, a stolon will grow one unit or not at all in each generation with a probability of 0.75.

We have tried to establish a set of generating rules which, when applied recursively, in the manner of Ulam's pattern-generating rules, generate a pattern that can be compared statistically to that of the Podocoryne carnea colony.

For each parameter a potential biological mechanism has been assumed, and this mechanism translated into terms that permit its being applied as a growth rule. Initially we have chosen to simplify the rules as much as possible, for what a cell might do instantaneously takes days of computation or minutes of computer time. For example, a cell might grow in proportion to the food available to it--in that sense it functions as an analog computer; to compute the food arriving at a point in a complex network via all possible paths is much more difficult, unless one can resort to an analog device such as a cell.

Some simplifications are less mathematical expedients than they are reflections of our ignorance of what the colony does and how it might do it. The attempt to synthesize colony growth has made explicit questions that must be answered in order to describe colony growth and in some cases has indicated that synthesis may require different specifications than description.

The Technique of Computer Simulation

This is an attempt to simulate on an electronic computer the development of the marine hydroid Podocoryne carnea as a colony is formed by a single hydranth placed on a microscope slide. The computer generates a ^{relatively} ~~relatively~~ complex pattern as a mathematical model of the growing system by iteration of a set of simple rules of growth. The model consists of a connected network of points in a two-dimensional mesh.

The rules of growth define the probability of growth for each stolon, and provide methods for choosing branching points

for new stolon formation and for inserting new nutritive hydranths on the growing stolons. The growth is effected in discrete amounts in a generation time, in the following sequence:

1. The probabilistic rule of stolon growth is applied to each stolon in turn, each generation, to determine whether or not it grows.
2. The rule for formation of new stolon branchings is applied, and new branch points and directions of branching are chosen.
3. The hydranth insertion rule is invoked, and the position of new hydranths recorded.

Finally, the growths, in effect, made. Some existing stolons grow, new stolons branch off, stolons anastomose by intersecting old ones, and new hydranths appear. The computer records this new connectivity in a manner comparable to plotting the new pattern on paper.

This constitutes a generation of growth. In the next generation the same rules are applied to the new configuration to determine future growth.

Each stolon terminus is tested each generation for growth.

The rule for stolon growth is defined as a probability. This probability that a particular stolon grows is a function of the number of hydranths in a connected neighborhood of the stolon terminus. The computer traces all connected paths in this neighborhood and counts the number of hydranths. The count is suitably normed so that it becomes a probability. The machine then "throws" with this probability to determine if the

stolon grows. This is done by generating a so-called "random number" and comparing it with the probability.

There is an algorithm for generating on the computer a very large set of numbers between zero and one, which have the statistical property of being "random." Each number in this set can be obtained from the previous number and an initial generator number. One property that this set must have is that the numbers in it be uniformly distributed in the interval from zero to one. Suppose, for example, that a stolon has a probability of growth = $1/5$. The computer then generates the next number in the sequence of random numbers. If the number is $\leq 1/5$ (less than or equal to $1/5$), the stolon grows. If the number is $> 1/5$ (greater than $1/5$), the stolon does not grow. This is equivalent to the "coin tossing" one might do to make this decision by hand.

The initial rule for adding new stolon branch points states that a branch point is added to the configuration whenever " \underline{x} " new points are added to the pattern and whenever a stolon anastomoses. In our runs we have chosen $\underline{x} = 4$. The branch point is chosen at random from the set of all points in the pattern. The machine again uses a random number to choose this point. That is, if there are \underline{n} points in the pattern, each point has probability = $1/\underline{n}$ of being chosen at the branch point. The direction of branching is also chosen at random. Once a branch point is chosen it is regarded as a new stolon terminus and this tested for subsequent growth according to the stolon rule.

Our hydranth insertion rule states that a new hydranth is added to the pattern whenever three adjacent (connected) points without hydranths can be found. Each generation the computer tests for hydranth insertion all new growth and all points where stolons have anastomosed this generation. (This has been varied to provide for hydranth insertion at the penultimate point whenever four adjacent connected points can be found.) The regions where stolons anastomose by intersecting other stolons form a higher connectivity of the pattern. Thus new hydranths can form in older regions of the growth. The hydranth insertion rule also attempts to preserve the constancy of the ratio of stolon length to hydranth number, which is observable in the laboratory development of the colony.

Another rule provides for formation of a second type of hydranth, the sexual or reproductive one, in regions that have high connectivity and consequently a high density of nutritive hydranths.

At the end of each generation various analyses of the pattern can be made and printed out. The stolon length to hydranth number ratio, spatial distribution of hydranths, branch point distribution, etc., can thus be compared generation by generation in the pattern built up. The pattern can also be plotted. For example, each generation of growth can easily be plotted by the machine and displayed on successive frames of microfilm.

It is evident that the pattern of growth could be played out by hand on graph paper, with coin tossing or tables of random numbers used to effect the probabilities. In fact,

locating the hydranths in a network to get probabilities is conceptually easier graphically than the method programmed for the computer. The obvious advantage of the computer is the number of generations and the size to which one can grow the pattern in a relatively short time. For example in a typical case using rather simple rules of growth, we could grow a pattern through some fifty generations with a total of some 500 occupied points in about three minutes of machine time on a high speed computer (IBM 7094). One can make slight changes in one parameter of a rule of growth and run a number of different cases in a relatively short time.

More complicated rules of growth can be used at the expense of more time spent computing. The arithmetic of evaluating a more complicated probability would not take much more computing time. The tracing of more and longer connected paths in a network, however, is time consuming. In particular, assume that the contribution of every hydranth to the growth of every stolon were known, and could be computed each generation along the shortest connected path in the network between the stolon terminus and the hydranth. This computation would be prohibitive in time in a few generations of growth, not to mention the complications of multiply connected paths between points.

The mathematical model consists of a network of points in a two-dimensional mesh. That is, all units of growth are constrained to be integral values of the mesh, and all branchings occur at right angles to the direction of growth. The stolon termini and the hydranths then occur only at mesh points, and

stolons intersect only at such points. These are, of course, rather severe limitations in the model. It was felt that initially such a simple model was more feasible for investigating different rules of growth and for observing how small changes in one rule could affect the patterns.

A so-called "non-mesh" code which would allow any angle of branching and any increment of growth in a generation could be written. Such a code [∞]would be considerably more complicated and would require much more machine time per run. The problems of representing connectivity and of determining whether stolons intersect are no longer trivial in a non-mesh code.

Biological Justification

The growth of individual stolons

If $d_{i\mu}$ is the minimum path length from the μ th hydranth to the i th stolon growth points, then $\sum_{\mu} \frac{1}{d_{i\mu} + 1}$ is the sum of the inverse of the distance from the point i to all hydranths along the minimum path length ($d_{i\mu} + 1$ is specified in order to avoid $1/d_{i\mu} = 1/0$, as it would be were we to consider a stolon point at which existed a hydranth) and represents the nutritive contribution of all hydranths to the growth of a stolon point.

Then $\sum_i \sum_{\mu} \frac{1}{d_{i\mu} + 1}$ is the summation of the nutritive contribution of all hydranths to all points in the colony.

The relative growth (f_i) of the i th stolon will be:

$$f_i = \frac{\sum_{\mu} \frac{1}{d_{i\mu} + 1}}{\sum_i \sum_{\mu} \frac{1}{d_{i\mu} + 1}}$$

and the absolute growth of the colony, (G) would be proportional to the amount of food in the colony, which in turn, assuming optimum conditions, would be a function of the number of hydranths in the colony.

Therefore:

$$G = (K) N$$

where G is the total growth expressed as stolon units, of the ($n + 1$)th generation, K is a constant of proportionality, and N is the absolute number of hydranths at the end of the n th generation.

Then the absolute growth of the i th stolon (G_i) will be:

$$G_i = (K) N \cdot f_i$$

In this matter it would be possible to translate a situation in which growth at a stolon point was dependent upon the amount of food arriving there through the stolon system in which imperfect mixing diminished a hydranth's contribution to a point in proportion to its distance from that point. The rule we have used simplifies the statement to consider only the hydranths in a circumscribed area irrespective of their distance within this area.

The assumptions implicit in this formulation are the following:

1. d_{ij} is taken as the minimum connected pathway between the i th stolon and the j th hydranth. Clearly the nutrient that reaches the j th hydranth is that arriving via all possible pathways, not only the minimum path length.
2. It is assumed that mixing within the stolon circulatory system is not perfect, and that the

amount of nutrient arriving at the i th stolon tip from the j th hydranth is some function of the distance. (Here $1/d_{ij} + L$)

3. It is assumed that growth of a stolon tip is proportional to the food arriving there, implying, in turn, that growth is the result of cellular multiplication at that stolon tip. Another possibility is that cells are formed elsewhere in the system and travel through the stolon to the growing tip.

Stolon Branching

A variety of mechanisms are consistent with what we know of stolon branching in real colonies. No systematic analysis of colony pattern to determine branch point geometry has as yet been carried out. We do know, however, that although any isolated hydranth will form stolons at its proximal end, hydranths in the colony, except for the primary one, do not originate stolons. We also know that more distal stolons can be induced in isolated hydranths by tying a fine hair around the body column, whereupon stolons will form just proximal to the hair.

If ^a stolon growth point produced an inhibitor of other growth points, and this inhibitor were constantly in excess of a growth threshold, then there would be only one growing stolon.

If a highly unstable inhibitor that diluted into the stolon ^{were} ~~was~~ continuously produced, then a maximum concentration would exist at the origin which would fall off further from that point; this would result in a distribution of branch points

tending to regularity. Gross observations on real colonies suggest that this regularity is not in evidence. The possibility that it exists but is masked by stolon branching and anastomosis cannot, however, be ruled out.

If a relatively stable inhibitor of branching were produced at a rate that balanced its destruction, or if a discrete amount of a stable inhibitor were produced once during the formation of a new stolon growth point, then the only way in which its concentration would be lowered would be by growth of the entire system. When through stolon growth the system became so large that the concentration of this inhibitor fell below a threshold, then any point in the system would be as likely to form a branch point as any other. With the formation of a new growth point, an additional quantity of the inhibitor would be injected into the system, and the formation of other points would be blocked.

This latter assumption is the one that has served as the basis for our programming of stolon branching.

The rule that is followed specifies that for every four units of stolon growth a new stolon growth point (branch point) is chosen at random from among the existing points, and a direction (up or down, left or right) is also chosen at random.

One problem arising in the application of this rule is whether stolon growth points that have anastomosed, and thus disappeared, should be replaced (by choosing in each generation, in addition to the new stolon growth points dictated by stolon growth in the previous generation, additional new growth points equal in number to those that have anastomosed). If the real colonies follow the former method, that is, non-replacement,

then it would seem unlikely that the stolon tip continuously produces a growth controlling factor. Colonies exemplifying both alternatives were generated, and will be considered below.

Hydranth Insertion

If stolon pieces differing from each other according to whether they do or do not bear hydranths are isolated from a colony by scraping the remainder of the colony away from the desired portion, the following results are obtained after two days (Braverman, unpublished). Ninety per cent of the time, buds appear on stolon^s originally containing no hydranths. On only twenty per cent of the stolons bearing either one or two hydranths do new buds appear (Table 1).

It seems that the presence of hydranths on a segment of stolon inhibits the appearance of other hydranths on that segment.

Our hydranth insertion rule attempts to reflect this inhibitory control by providing that a hydranth will be inserted at a point if the point is one of three adjacent connected points without hydranths. Points of the n th generation are considered for hydranth insertion, as well as older points that are joined by newly formed stolons. Some later colonies are programmed with a similar rule, with the exception that four adjacent points are required, and hydranths are inserted on points of the $(n - 1)$ th generation.

It is clear that on a straight stolon either rule would result in a regular pattern with a hydranth every three units (Fig. 7a). The interhydranth distance of hydranths on the

straight line colony would remain at 3.0. With increasing connectivity, the pattern of Figure 7b might, however, result. The rule determining connectivity has a strong influence on hydranth distribution (Fig. 7c). The effect of varying connectivity on the interhydranth distance will be discussed below.

It is clear that if control by an inhibiting substance is involved, then our rule for scanning four adjacent points and inserting a hydranth at the $(n - 1)$ th point is but a crude approximation to a possible biological mechanism. A more precise representation of the amount of inhibitor arriving at a point would take the form proposed for the arrival of the hydrantha's nutritive contributions to a point; that is, it would represent the summation of inhibitor arriving from all hydrantha's at a point, via all possible pathways, modified by a factor representing attenuation of the inhibitor due to time and distance travelled (i.e., the lability of the substance, and the amount of it removed from the coenosarc by tissues along the way), and by the nature of mixing within the stolon circulation. Although preliminary analysis indicates a tendency for new hydrantha's to be formed on stolons of the $(n - 1)$ th generation (that is, on the previous day's stolons), there appears to be a small consistent deviation from this tendency: hydrantha's also form on older stolons and on stolons of the n th generation. An investigation of the mechanism controlling hydranth formation must wait for a clear understanding of the time sequence, and geography of hydranth insertion on stolons. Cell division kinetics and the dynamics of cell movements within the system may be relevant to this question.

Sexual Zooid Insertion

Sexual zooids are formed de novo (for the larger part) in real colonies, and generally are first seen in the immediate vicinity of the initiating hydranth. The sexual determination is limited to localized areas in the stolon mat (Braverman, 1963). Age or density could be the responsible factors for the initiation of this second type. Either factor could operate by reducing the opportunity for equilibration of such metabolic products as carbon dioxide which is known to stimulate precocious and adventitious medusa formation (Braverman, 1963).

Our initial code operates via density. When the count of nutritive hydranths exceeds a determined number within a specified neighboring area, we mark the point as a red point. Red (sexual) zooids can subsequently form within a specified radius of this point if they satisfy the same rules as pertain to black hydranths, i.e., no hydranths within a specified path length. We have constructed colonies following rules in which red and black are either ignorant of each other, or we have included both red and black in determining the possibility for hydranth insertion. We have also programmed red zooids to form as a function of stolon age, using as a trial age the average generation at which red zooids first appear. Although there is a slight tendency for red hydranths to form closer to the center using these rules, the two sets of rules are essentially equivalent in their results.

The rate of sexual zooid formation, the percentage of sexual zooids, the tendency for this percentage to stabilize, for the rate to increase or decrease, and the radial distribution

of sexual zooids--each of these parameters varies with quantitative variation in the rules, and not always in a manner that could be predicted a priori.

Colony Generation

The rules generating the first set of colony patterns are as follows:

1. Two-dimensional mesh, growth restricted to mesh points.
2. Stolons can grow in four directions from the original point.
3. The unit of stolon growth is invariant.
4. Once established, a stolon grows in a straight line.
5. A potential growth point grows with a probability dictated by the count of hydranths within all connected path lengths of five units from the point. If this count ≥ 5 , deterministically grow one unit in the next generation. If the count < 5 , then $1/5 \cdot (\text{count}) =$ probability of growth.
6. Insert hydranths at points of the nth generation if there are three adjacent points without hydranths.
7. For every four new points added, and for every time a stolon terminates, choose a new branch point at random.
8. If the count mentioned above (rule 5) is ≥ 6 , mark this point as a "red point." Subsequently, if one can insert any more hydranths within a connected path length of three units from this point, mark these as "red" or reproductive zooids. Such zooids do not count in any probabilities of growth.

Programmed with rules such as these, the computer carries out the indicated operations and describes the state of the colony after each generation. It may also be programmed to carry out a number of analytical operations. The print-out for any generation consists of:

1. A list of the occupied stolon points, identified by their position on a two-dimensional matrix, the direction in which the stolon grew to each point, and a description of the connectivity at each point specifying which of the fifteen possible configurations of lines about a point, in four directions, exist at that point.

2. A list of hydranths, also identified by co-ordinates; the red hydranths (sexual zooids) are identified in this list by an added symbol.

3. A list of all red points.

4. A list of all branch points, and the direction in which they branched.

5. A list of the potential branch points that are to be considered for growth in the next generation.

6. A list of dead branches, that is, stolons that have been removed from consideration for growth because they have anastomosed.

7. A list of the branches that will die at the end of the present generation.

8. A list of the possible growth points that are not growing in the next generation.

9. A list of the possible growth points that are growing in the next generation.

10. An analysis of the radial distribution of black and red hydranths separately.

11. An analysis of the frequency of hydranth distribution among the three possible interhydranth distances (one, two or three stolon units) expressed as percentages.

12. A statement of the number of entities in each of the above categories, a statement of the percentage ^{of} black and red hydranths, the ratio stolon length/hydranth number, and the generation number.

Problem No. 3, generated by these rules (Fig. 8) is a pattern of high connectivity. Seventy percent of all stolon points are branch points (Table 3). A small number of isolated stolons with an interhydranth distance of three exist, but for the larger part stolons branch soon after they are formed. At the end of the run of thirty-four generations, four per cent of the hydranths were three units apart, twenty-eight per cent were two units apart, and sixty-eight per cent, one unit apart (Fig. 9). Due to the high connectivity, generative zooids, represented in Figure 8 by circles, comprised fifty-nine per cent of all zooids, and their proportion appeared to be increasing (Table 3).

Two phases of stolon growth can be recognized in this pattern (Fig. 10). The first ends with the appearance of generative zooids in the colony. Thereafter stolon growth proceeds at a constant rate, despite the fact that the rate of live stolon increase (i.e., the total stolon points minus those that have terminated through anastomosis) is decreasing.

A high percentage of the stolon growth points live only one generation, thus the long stolon lines represent anastomosis of many separate stolons, rather than the growth of a single one.

If the rule for the formation of red points is changed to require that the count of black hydranths within a patch length of five should be seven instead of six (Tables 2 and 3, Fig. 11) there is little reflection of this change in any parameter of colony growth other than the proportion of red hydranths. (Note, the identity of the two colonies up to the time the new red hydranth rule is invoked is due to their being generated with the same random number series.) The slope of the red hydranth curve (Fig. 11) suggests that the tendency in this problem, No. 6, as in No. 3 (Fig. 10), is for the proportion of red zooids to increase.

The same holds true if the rule is changed to specify ten black hydranths in the neighborhood before a red point is established (No. 8, Table 3). Although the percentage of zooids after thirty-two generations is reduced to thirty-five, their rate of formation is increasing.

Apparently quantitative variation in the specification of the red point rule exerts little influence upon the ultimate colony form.

When the drastic revision of the rule specified for Problem No. 10 (Table 2) was programmed, and run with the same random number sequence as Nos. 3, 6, and 8, there was still little change (Table 3, Fig. 12).

If the replacement rule is changed, however, profound differences in colony morphology occur. Generated colonies Nos. 5, 7, and 12 (Tables 2 and 3) utilize a modification of this rule which specifies that the new branch points in each generation will be equal to $k/4$ (where k is the number of stolon points added in the previous generation). No provision for replacement of stolons terminating in anastomoses was made.

The colony pattern resulting from this change in the replacement rule (Fig. 13) is far more open than that in which dead stolons are replaced (Fig. 8). Problem No. 12 grows more slowly (Table 3) than No. 3, occupying only 166 points in thirty generations, compared to 255, but it covers more area in doing so. Problem No. 3 almost completely fills in the area it occupies, whereas No. 12 fills in the mesh irregularly--except for the center, which at thirty-four generations is fairly well filled. Sexual zooids (circles) follow density. In No. 3 they are ubiquitous in the colony; in No. 12 they are more limited to the central area.

At thirty-four generations (Fig. 10) the growth rate of No. 3 appears to be constant and the sexual zooids, which appeared in the colony at about generation fifteen, are increasing in proportion. Sexual zooids do not appear in No. 12 until after generation twenty (Fig. 14) and their numbers at thirty-four generations are so few that no tendency in their rate of increase can be abstracted.

The rate of stolon growth and that of hydranth formation, however, appears to be constantly decreasing between generations one and thirty-four. By sixty generations (Fig. 15) this tendency is clear cut. The ratio of points of the n th generation

at sixty. The growth rate appears to be falling off to a point at which growth will be constant. This appears to be true of Nos. 5 and 7, each of which was generated with the no replacement rule.

Discussion

The sample colonies generated up to this date (i.e., up to Problem No. 12) are largely of an exploratory nature. They represent the first attempts to test whether the code we have written satisfactorily copes with all the problems that arise during the course of colony generation. Thus far, our major conclusions relate to the paucity of operational rules that are required to generate a pattern of such complexity.

In respect to making explicit the required operations, the exercise of computer generation has served to specify exactly what parameters of colony growth we must be cognizant of in order to program the operations of the growing colony. Our future plans for refining the growth rules are dependent on acquiring more information about how real colonies grow-- not only information regarding the pattern of the colony but also information as to how an operation is carried out, e.g. of what the hydranth insertion rule consists. The pattern of future research that we contemplate takes the form of a continuous dialogue between questions and suggestions arrived at through computer generations, and biological experiments and observations answering and proving these.

These initial generated patterns have mainly served to define for us the parameters that are of sufficient interest

to warrant subsequent investigation in greater detail. Authoritative comment upon the effects of varying a rule will not be appropriate until the same rules are run a sufficient number of times (with a different random number sequence each time) to establish the statistical significance of the resulting pattern.

Although we have been satisfied, in our initial attempts, to define stolon growth with the crudest approximation to a biological mechanism, we are now attempting to devise a workable algorithm to reflect a somewhat more realistic appraisal. The ideal formulation summarizing the influence of each hydranth in the colony upon each potential growing stolon point, is computationally unfeasible at the present time; it is reasonable, however, to analyze the influence of the hydranths within a circumscribed neighborhood, and test the assumption that contributions from hydranths external to the neighborhood are insignificant by comparing patterns generated with significant neighborhoods of varying sizes.

In the model proposed, the relative growth of the ith stolon $f_i = \frac{\sum_n \frac{1}{d_{in} + 1}}{\sum_i \sum_n \frac{1}{d_{in} + 1}}$ is transformed into absolute growth by specifying that $g = (K) N$, the total stolon growth in the colony is a function of the number of hydranths, and that $(g_i \in (K) N \cdot f_i)$ the absolute growth of the ith stolon is proportional to the number of hydranths in the colony and the relative growth of the ith stolon. Stolon growth should therefore be exponential, since the number of hydranths is a

function of the size of the stolon system. Somewhat surprising is the strong tendency to exponential growth in the problems already generated, since their generating rules in no way provide for total growth as a function of the existing colony size. Apparently hydranth proximity is closely enough related to colony size so that exponential growth is obtained, even with the present rules. It should be emphasized that the rules utilized up to the present time, contain no explicit strictures regarding the rate of colony growth, yet the growth rate, in colonies following the ~~exclusion~~^{replacement} rule, tends to be constant.

The hydranth insertion rule, too, represents a crude approximation to a biological mechanism, specifically that recognizing the influence of existing hydranths on hydranth formation. Better approximations of the order of that suggested for the stolon growth rule are contemplated, as are experimental proofs with real colonies of those control mechanisms that computer generation indicates are able to generate the observed hydranth patterns.

We are not as yet extracting from our computer generated colonies the maximum available information. With a small expenditure of computer time it is possible to analyze generated colonies for virtually any parameter. For example we could determine for each generation the average age of all extant hydranths or record the distribution of ages of stolons on which hydranths appeared. We are not yet sure what the significant parameters are, nor can we tell which phenomenon is directly specified and which is an unprogrammed consequence of a controlled operation.

It now appears feasible to consider a discrete grid approximation of the pattern of a real colony, so that the real colony growth can be described in our code and submitted to the computer for analysis that would be extremely tedious if carried out by "hand." Although the need for certain analyses is quite clear, there remains a reasonable doubt as to what other information it would be fruitful to extract.

Beginning with a set of rules designed to grossly approximate possible biological control mechanisms, we have generated a number of patterns. The growth of the patterns, even in analogy, differs in a number of respects from what we know of the growth of the hydroid. That is to be expected. It is, in the end, the specific rules that will determine the similarity or difference. The patterns generated by the recursive application of the rules, if not identical to the pattern of the growing hydroid, Podocoryne carnea, are of the same kind. Reiteration of the simple rules, in toto, in each generation, generates a pattern not only of increasing complexity, but incorporating new entities. The overall picture of a pattern of concentric differentiation extending peripherally, and the introduction of new entities, centrally, can be simulated as a function of the interaction of simple rules.

A point by point analysis of the Podocoryne colony describes a pattern of great complexity. This complexity need not, however, stand in a one-to-one relationship to the rules which generate the colony--that is, with the colony's genetic endowment. The computer generated colonies demonstrate that the same kind

of end product complexity can arise through the interaction of a small number of simple rules pertaining to the operations rather than the objects of development.

The developing hydroid colony is an extremely simple example of a self-organizing system, and the computer generated colony incorporates only a portion of the known parameters of colony variability. Even so, the relative success of this simple model suggests the possibility "that a relatively small number of instructions of a high power are capable of shaping the extraordinary complexity of animal organisms" (Hawkins, 1964). The current model for biological replication, involving the presence of a tape describing the organism, derives from von Neuman's (1956) work on the Turing machine, that is a machine that can make another machine identical to itself. This, however, is a machine springing full grown into the world-- perhaps a satisfactory model for bacterial replication, but not a satisfactory analogy to the animal which undergoes development.

The tape for this kind of machine--a developing machine-- would not be likely to contain a description of the machine at all, but rather would incorporate, among some descriptive elements, a series of simple interacting specifications which are applied to the changing environment they create in the egg, in such a way that the adult is the only possible consequence.

This idea is certainly not new. The view of bone as a developing system acquiring specificity from its environment

was suggested by Jeffries Wyman (1849) and considerable evidence regarding the formation of trabecular patterns in response to environmental stress exists (Murray, 1936). Gustafson and Wolpert (1963) come to the conclusion as a result of their painstaking studies on the morphogenesis of the sea urchin that the complex morphological events in early sea urchin development can be reduced to variations of a few basic cellular activities, in particular cell contact and pseudopod formation.

The theme of interaction among the shaping forces of the developing organism is explicitly formulated in D'Arcy Thompson's On Growth and Form (1963):

" . . . modifications of form will tend to manifest themselves not so much in small and isolated phenomena, in this part of the fabric or that, in a scapula for instance or in a humerus, but rather in some slow, general, and more or less uniform or graded modifications, spread over a number of correlated parts, and at times extending over the whole or over great portions of the body."

Although it would be a satisfying tour de force to generate a synthetic colony that is statistically identical to the real colonies, that is not our major goal. We are primarily interested in using a model system defined by a series of rules to investigate the dynamics of interaction among recursive rules, to develop techniques for the synthetic analysis of biological interaction, to explore the variance introduced into

the end product by changes in the generating rules, to explore the possibility that such rules, iterated in toto in each generation could generate an endogenously size limiting system and maintain a dynamic equilibrium in that system within a range of, externally originated, deviations.

None of these somewhat optimistic goals would be meaningful, however, without a rigorous demonstration that the model is capable of simulating the pattern and function of a biological system. For that reason we shall continue to investigate simultaneously the real and synthetic colonies in order to demonstrate conclusively that the actual mechanisms of development are translatable into generating rules.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

- Fig. 1. Pedocoryne carnea colonizes the shell of a hermit crab with nutritive, spiral, and generative zooids; the latter form gamete-bearing medusae. The egg, fertilized in the sea, develops into the planula which metamorphoses into a nutritive hydranth on the snail shell.
- Fig. 2. Average growth of four colonies of Clone W, grown at 23°C. σ stolon length in arbitrary units,
○ hydranth number.
- Fig. 3. The frequency distribution of interhydranth distance in colonies consisting of fifteen hydranths \odot and forty-five hydranths ○ .
- Fig. 4. A diagram of colony development over a four month period. Only two tentacles are shown on each zooid, and the size of the zooids is exaggerated.
- Fig. 5. A pattern generated by the recursive application of a simple rule.
- Fig. 6. The rule of Fig. 5 carried to twenty-three generations.
- Fig. 7. a. Application of this hydranth insertion rule to a straight stolon results in hydranths (\odot) three units apart. The next hydranth will be inserted at the point of the (n-1)th generation.
b. The point indicated by the arrow is eligible to receive a hydranth as there are three points lacking hydranths around it, and it is a point of the (n-1)th generation.
c. When the same configuration occurs at a point of the (n-2)th (or older) generation, the point is eligible for hydranth insertion.

- Fig. 8. The colony pattern of problem No. 3 ; nutritive hydranth, generative zooid.
- Fig. 9. The percentage of hydranths at distances of one, two, and three stolon units during the course of growth of problem No. 3.
- Fig. 10. The growth of problem No. 3 ; total stolon points, total hydranths, red (generative) zooids, live stolon points.
- Fig. 11. The growth of problem No. 6 ; total stolon points, total hydranths, red (generative) zooids.
- Fig. 12. The growth of problem No. 10 ; total stolon points, total hydranths, red (generative) zooids.
- Fig. 13. The colony pattern of problem No. 12 ; nutritive hydranths, generative zooids.
- Fig. 14. The growth of problem No. 12, thirty four generations ; total stolon points, total hydranths, red (generative) zooids.
- Fig. 15. The growth of problem No. 12, sixty generations ; total stolon points, total hydranths red (generative) zooids.

TABLES

Table 1

Hydranth Growth on Isolated Stolons

Exp. No.	No. of hydranths on stolon.	No. of cases.	No. of stolons bearing new hydranths.	Percentage of stolons bearing new hydranths.
1	1	5	0	0
	2	2	0	0
	0	8	7	87
2	1	8	3	37
	10	17	11	63

Table 2

Variations in Rules of Generated Colonies

Problem No.	Rule Five	Replacement Rule
3	As stated	Yes
5	As stated	No
6	Path length 5, 7 to form red pt.	Yes
7	Path length 5, 7 to form red pt.	No
8	Path length 5, 10 to form red pt.	No
10	Where $h =$ no. of black hydranths within path length of 5: if $0 < h < 5$ grow one or no units if $5 < h < 10$ grow two units if $10 < h$ grow three units, mark as red pt.	Yes
12	As 10	No

Table 3
Summary of Generated Colonies

Prob.No.	Rule 5	I.H.D. (30)	% Red	% B.P.	V+W (150)	T.P. (30)	$\frac{W_n + W_{n-2}}{2}$ $\frac{W_{n-10} + W_{n-12}}{2}$
Replacement							
3	6/5	54 40 6	59	70	37	255	2.55 (34 gen.)
6	7/5	68 25 7	46	62	39	267	3.05 (34 gen.)
8	10/5	49 48 5	35	66	35	326	3.15 (32 gen.)
10	10/5 var.	61 32 6	35	70	45	447	6.0 (30 gen.)
No Replacement							
5	6/5	34 38 28	30	26	23	98	1.0 (56 gen.) 1.71 (30 gen.)
7	7/5	35 41 24	26	26	24	118	1.24 (52 gen.) 1.87 (30 gen.)
12	10/5 var.	42 40 18	16	25	26	166	1.06 (60 gen.) 1.58 (30 gen.)

I.H.D. (30): Frequency distribution of interhydranth distance at the thirtieth generation, expressed as percentage.

% Reds: The percentage of all zooids that are red (or generative) zooids after the last generation.

% B.P.: The percentage of all stolon points that are branch points after the last generation.

$\frac{V+W}{(150)}$: The number of terminal stolon points (i.e. growing stolon points) in the colony when there are a total of 150 stolon points.

$$\frac{W_n + W_{n-2}}{2}$$

W are all the stolon points that grow in a given generation.

$$\frac{W_{n-10} + W_{n-12}}{2}$$

$\frac{W_n + W_{n-2}}{2}$ is the number of points that grow during the last generation and the (n-2)th generation averaged so as to counteract the effect of chance variation in a single

Table 3 (cont.)

generation. This figure gives an indication of the tendency of growth in a generated colony during the last ten generations.

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EXPLANATION OF PLATES

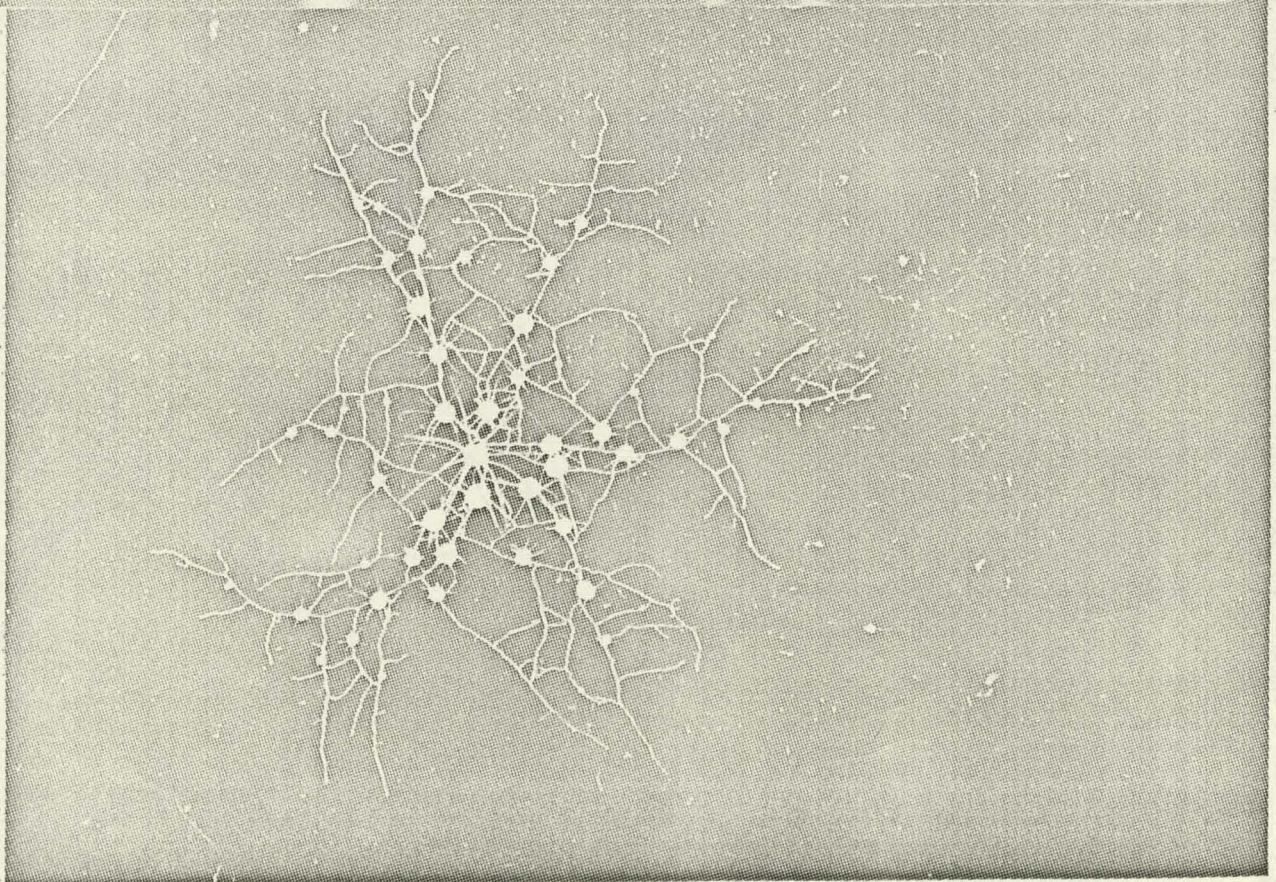
Plate 1

Fig. 1 A two to three month old Podocoryne carnea colony growing on a microscope slide. The colony consists of stolons, nutritive hydranths, and generative zooids (arrow).

Fig. 2 One of the daily photographs used to obtain data on colony growth.

FOOTNOTES

page 1: ¹ Work performed in part under the auspices of the Atomic Energy Commission.



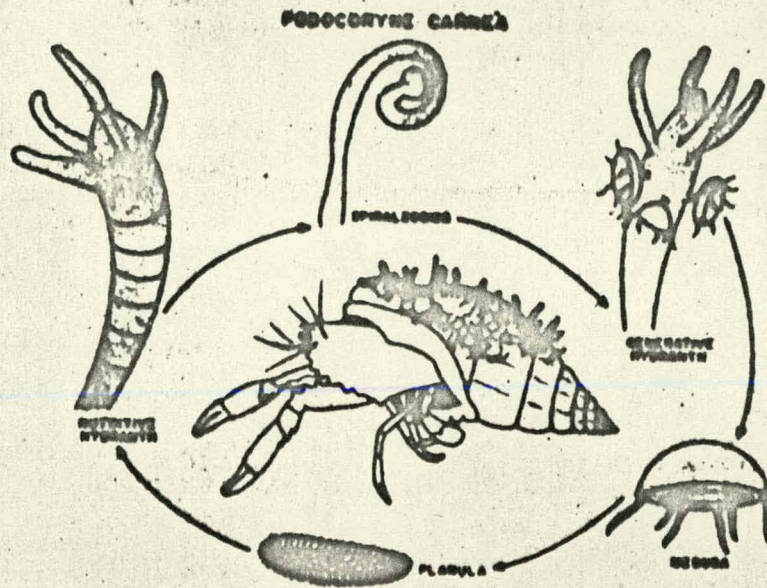


Fig. 1 Podocoryne carnea colonizes the shell of a hermit crab with nutritive, spiral, and generative zooids; the latter form gamete bearing medusae. The egg, fertilized in the sea, develops into the planula which metamorphoses into a nutritive hydranth on the snail shell.

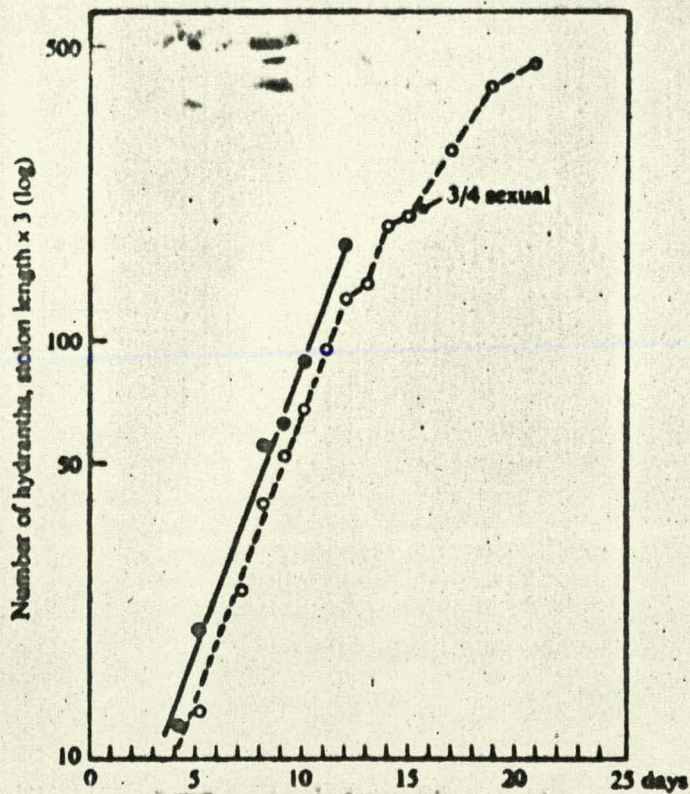
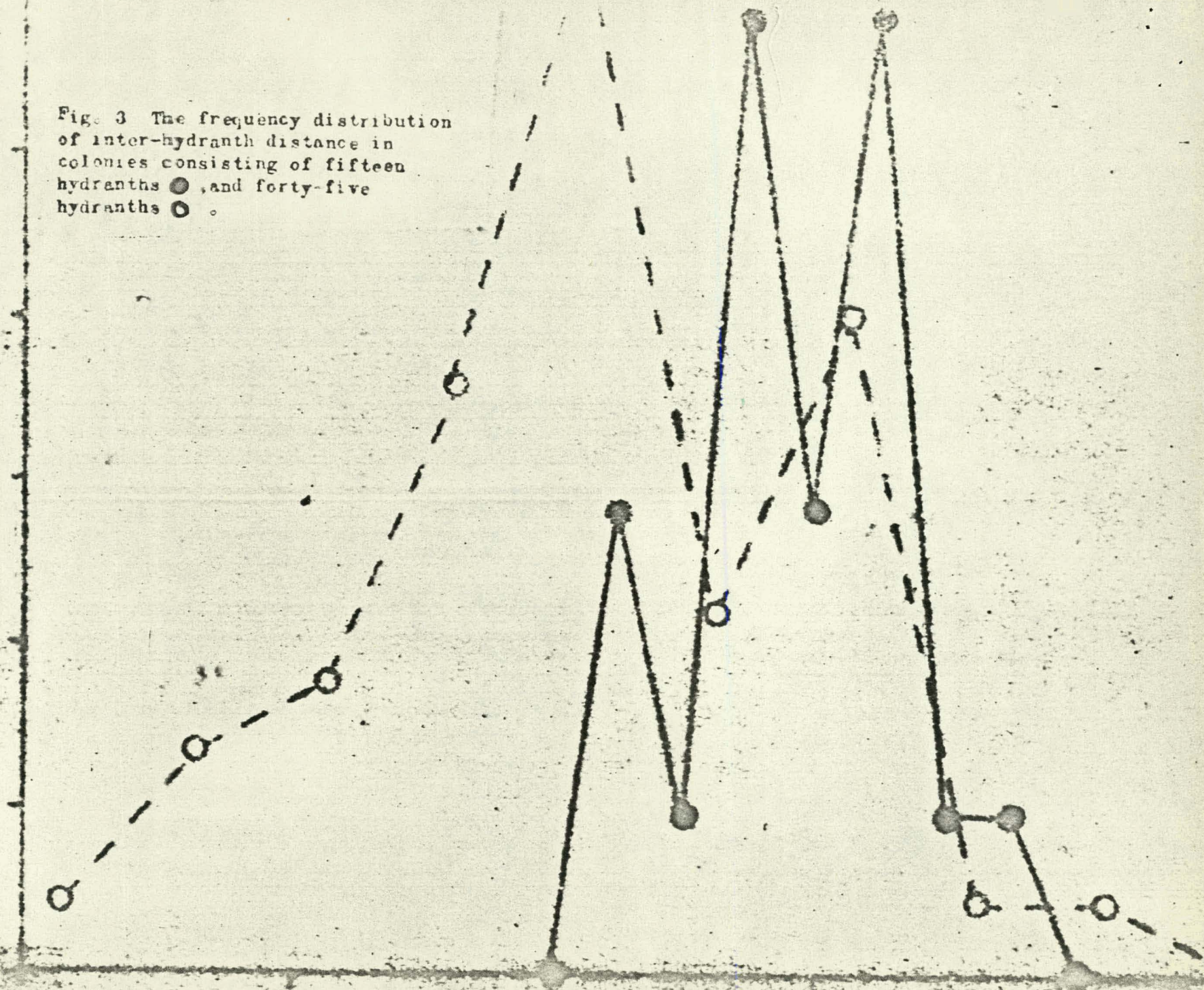


Fig. 2 Average growth of four colonies of Clone W, grown at 23°C. ● stolon length in arbitrary units, ○ hydranth number.

Fig. 3 The frequency distribution of inter-hydranth distance in colonies consisting of fifteen hydranths ●, and forty-five hydranths ○.



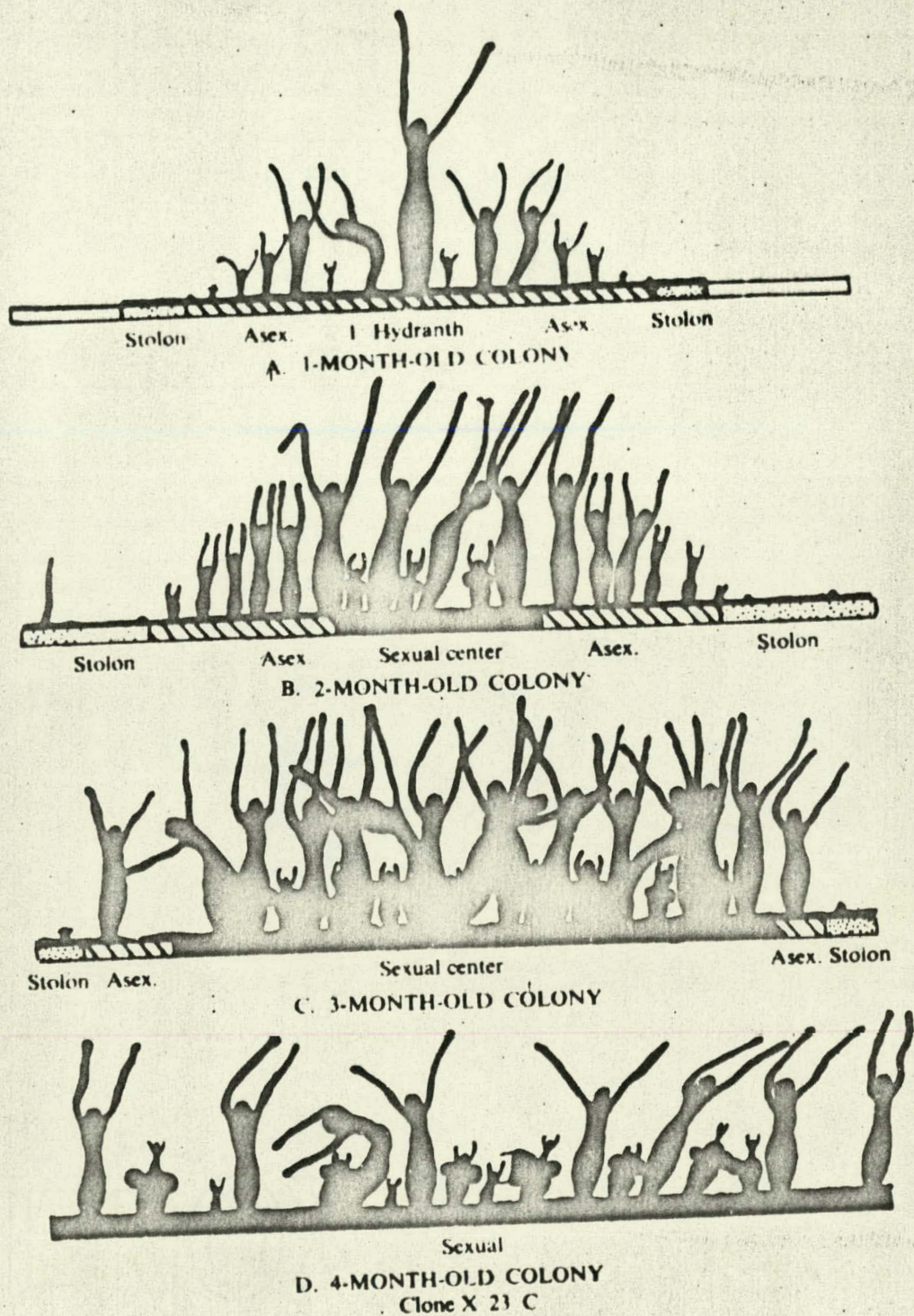


Fig. 4 A diagram of colony development over a four month period. Only two tentacles are shown on each zooid, and the size of the zooids is exaggerated.

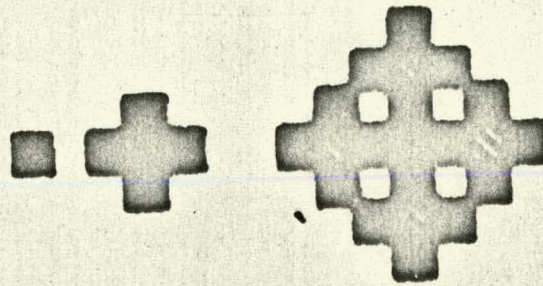


Fig. 5 "Given a number of squares in the nth generation the squares of the n+1th generation will be all those which are adjacent to the existing ones except that the squares which are adjacent to more than one square of the nth generation will not be taken." (Ulam, 1962)

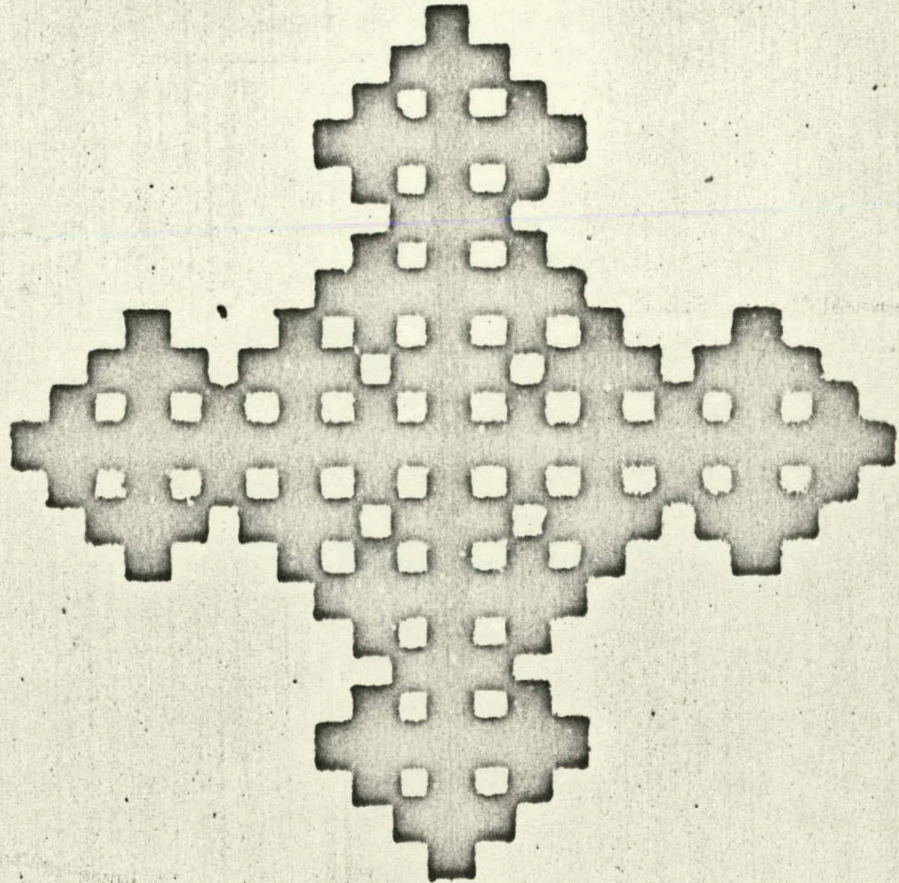


Fig. 6 The rule of fig. 5 carried to twenty-three generations.

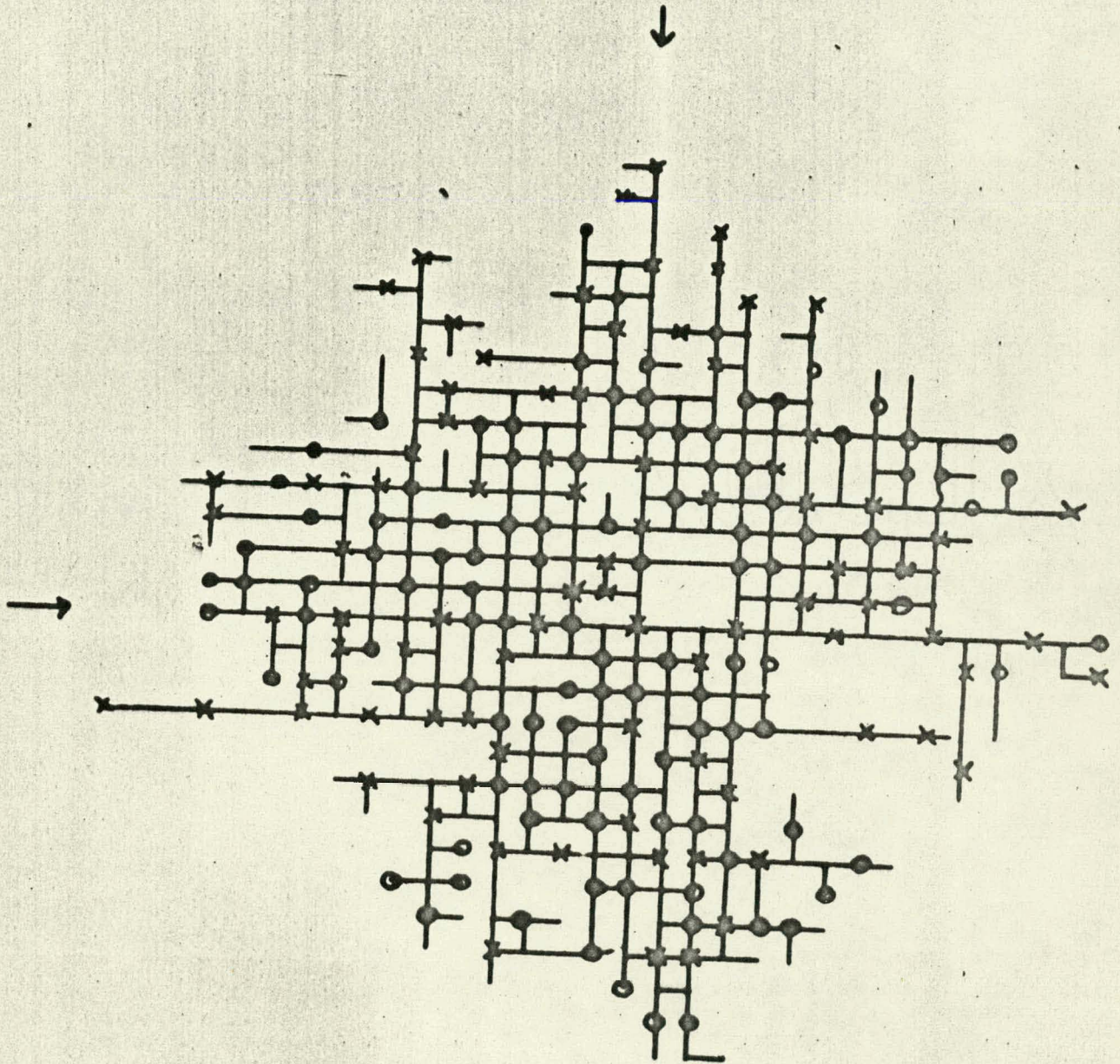


Fig. 8 The colony pattern of problem No. 3. X nutritive hydranth, O generative zooid

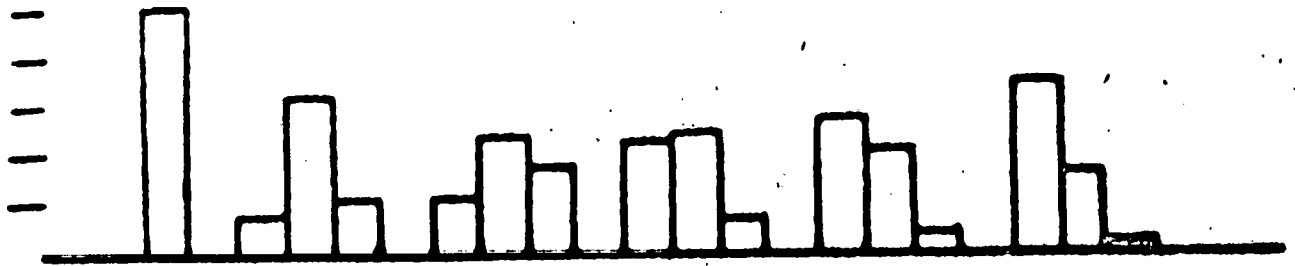


Fig. 9 The percentage of hydranths at distances of one, two, and three stolon units, during the course of growth of problem No. 3.

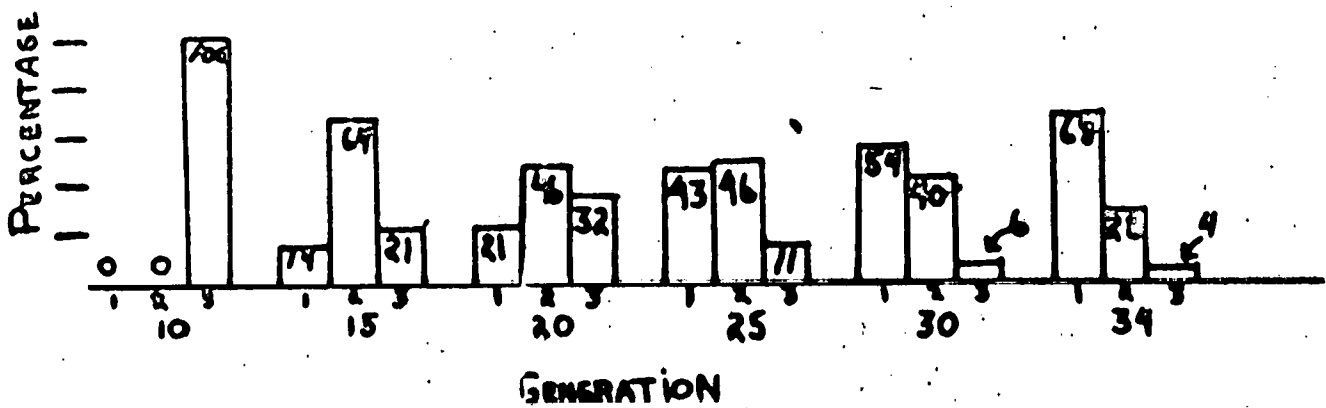


Fig. 10 The growth of problem No. 3. ● total stolon points, ○ total hydranths, X red (generative) zooids, □ live stolon points.

500

100

0

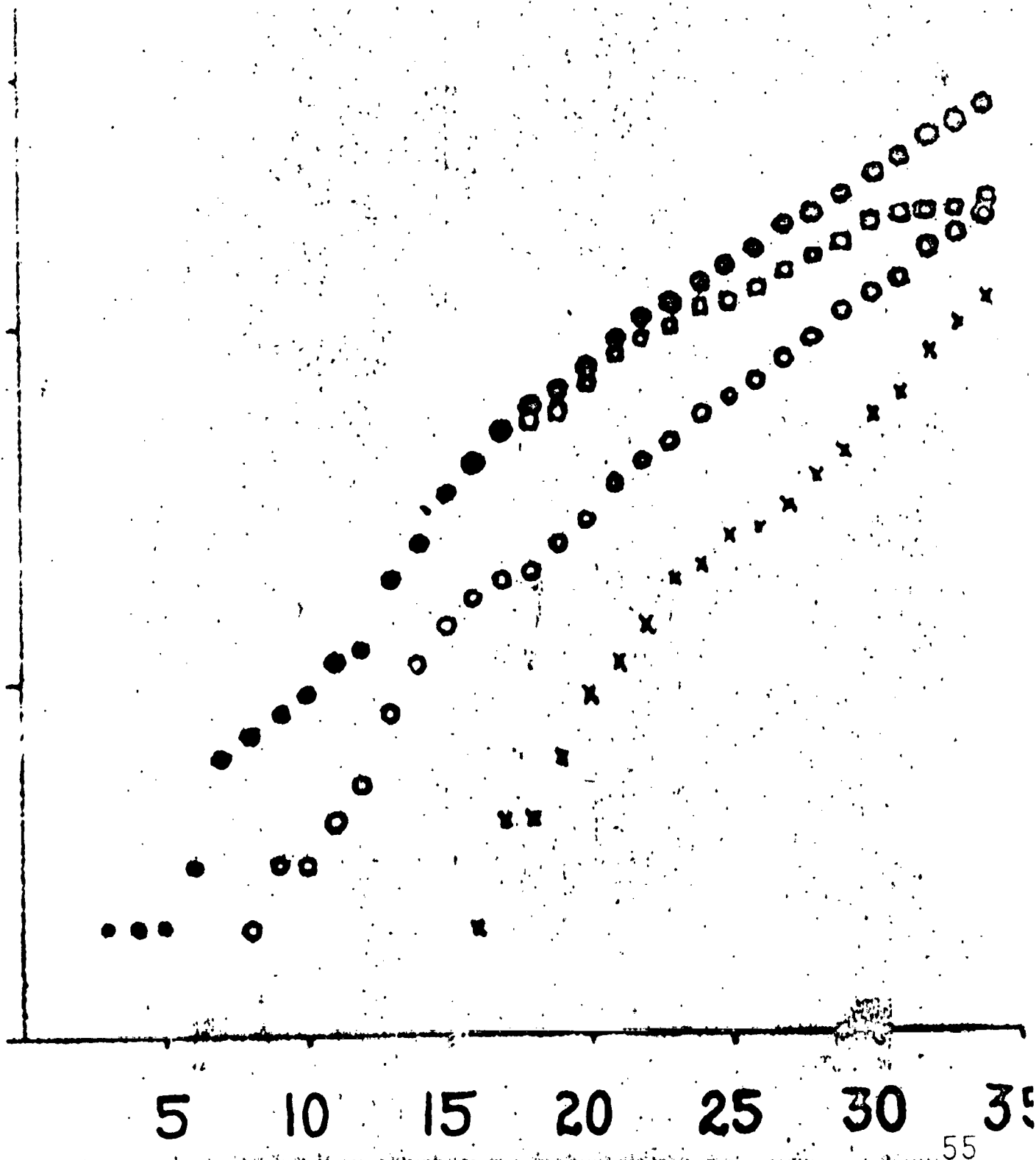


Fig. 11 The growth of problem No. 6. ● total stolon points, ○ total hydranths, X (subgenerative) zooids.

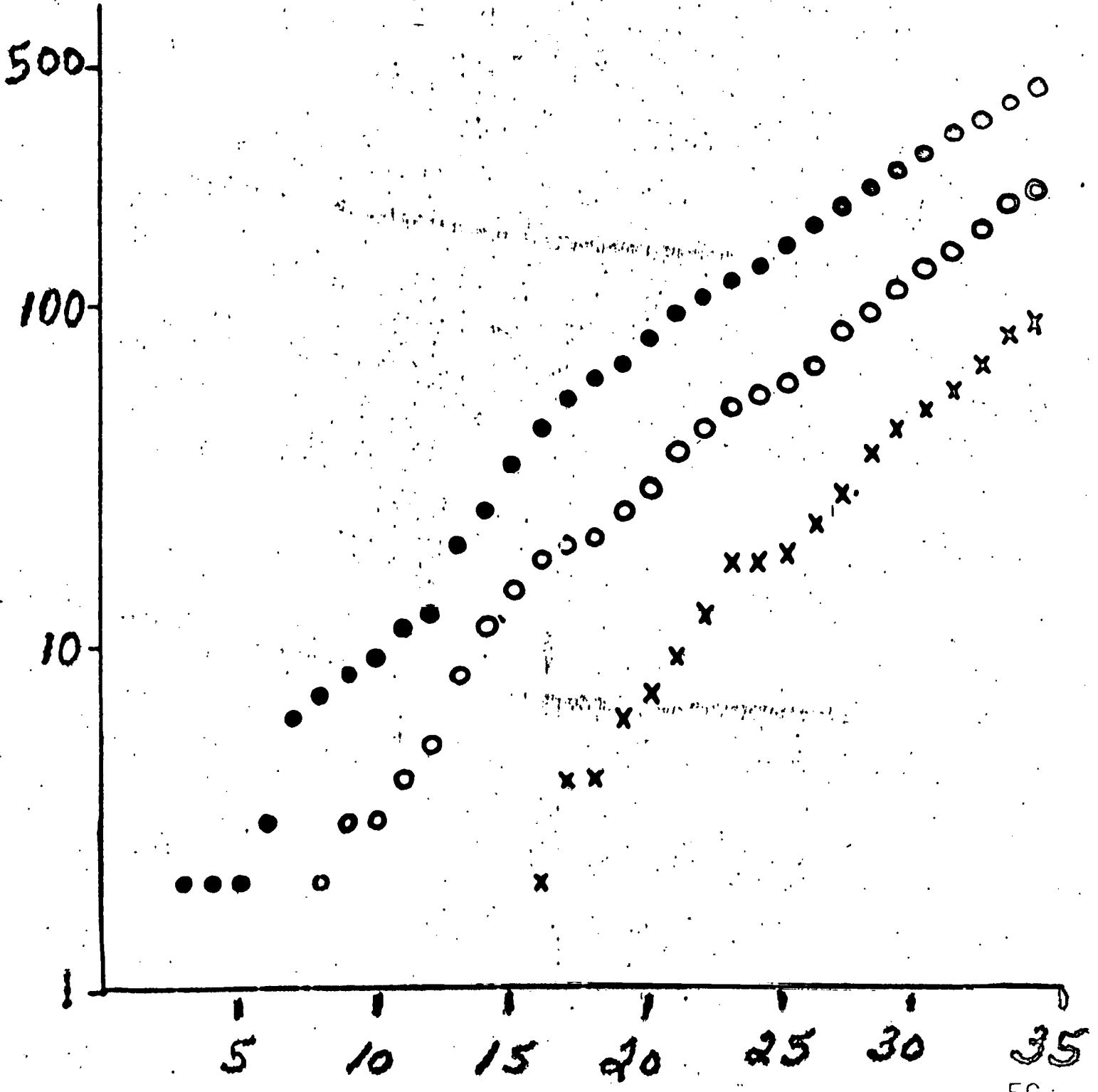
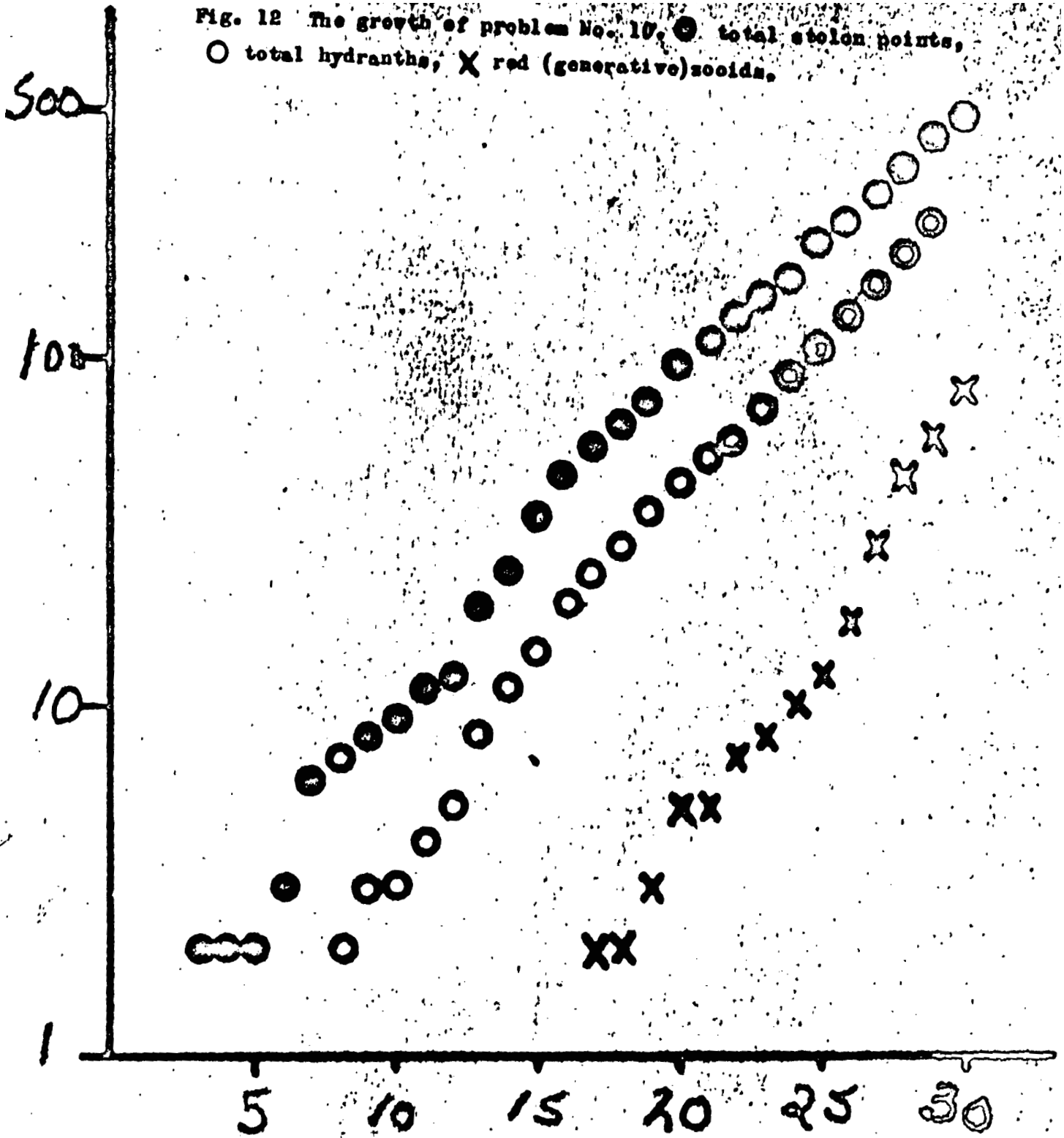


Fig. 12 The growth of problem No. 10. ● total stolon points,
 ○ total hydranths, X red (generative) zooids.



Run 10

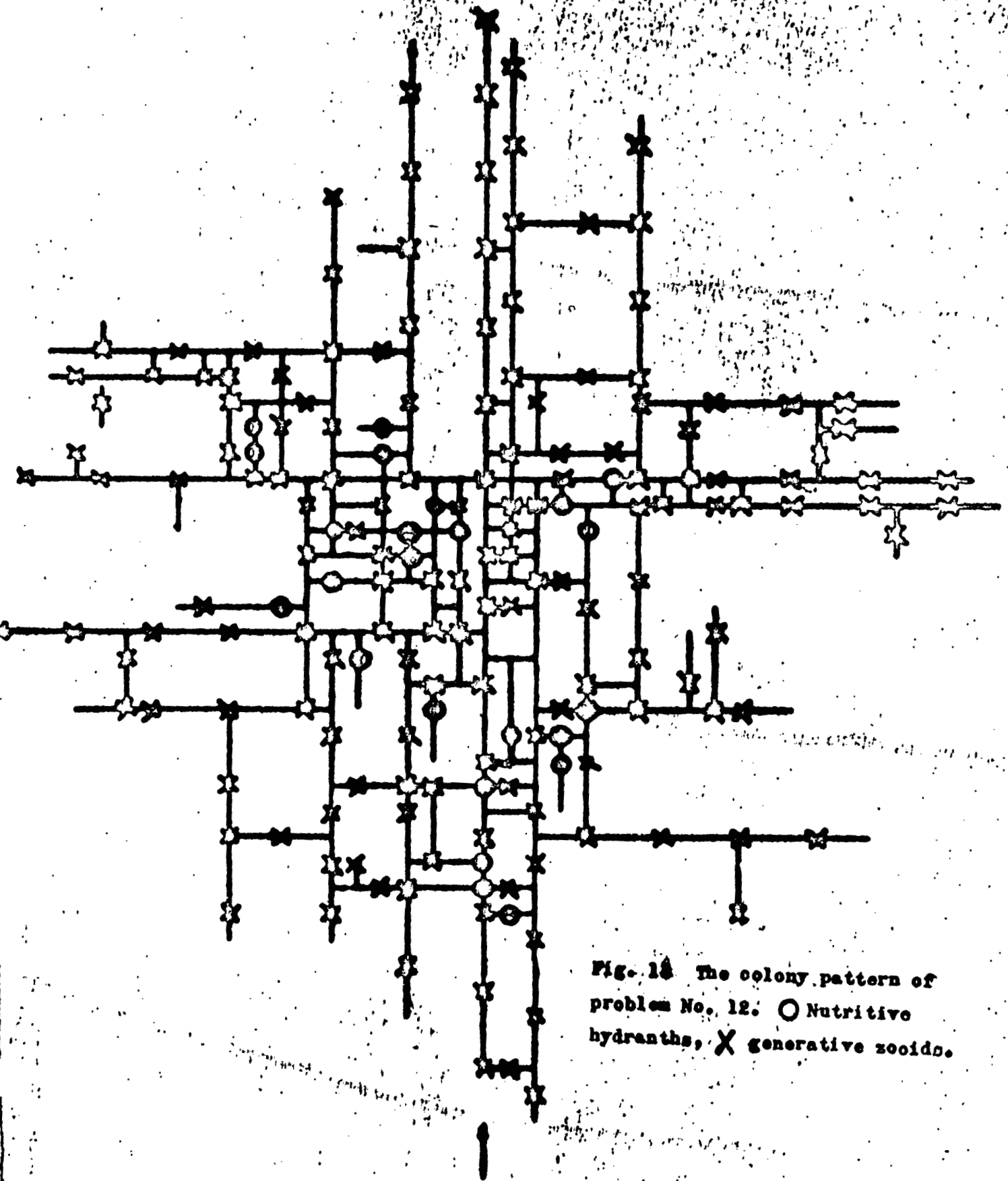


Fig. 18 The colony pattern of
problem No. 12. ○ Nutritive
hydranths, X generative zooids.

Fig. 14 The growth of problem No. 12, thirty-four generations.
 ○ total stolon points, ○ total hydranths X red (generative) zooids.

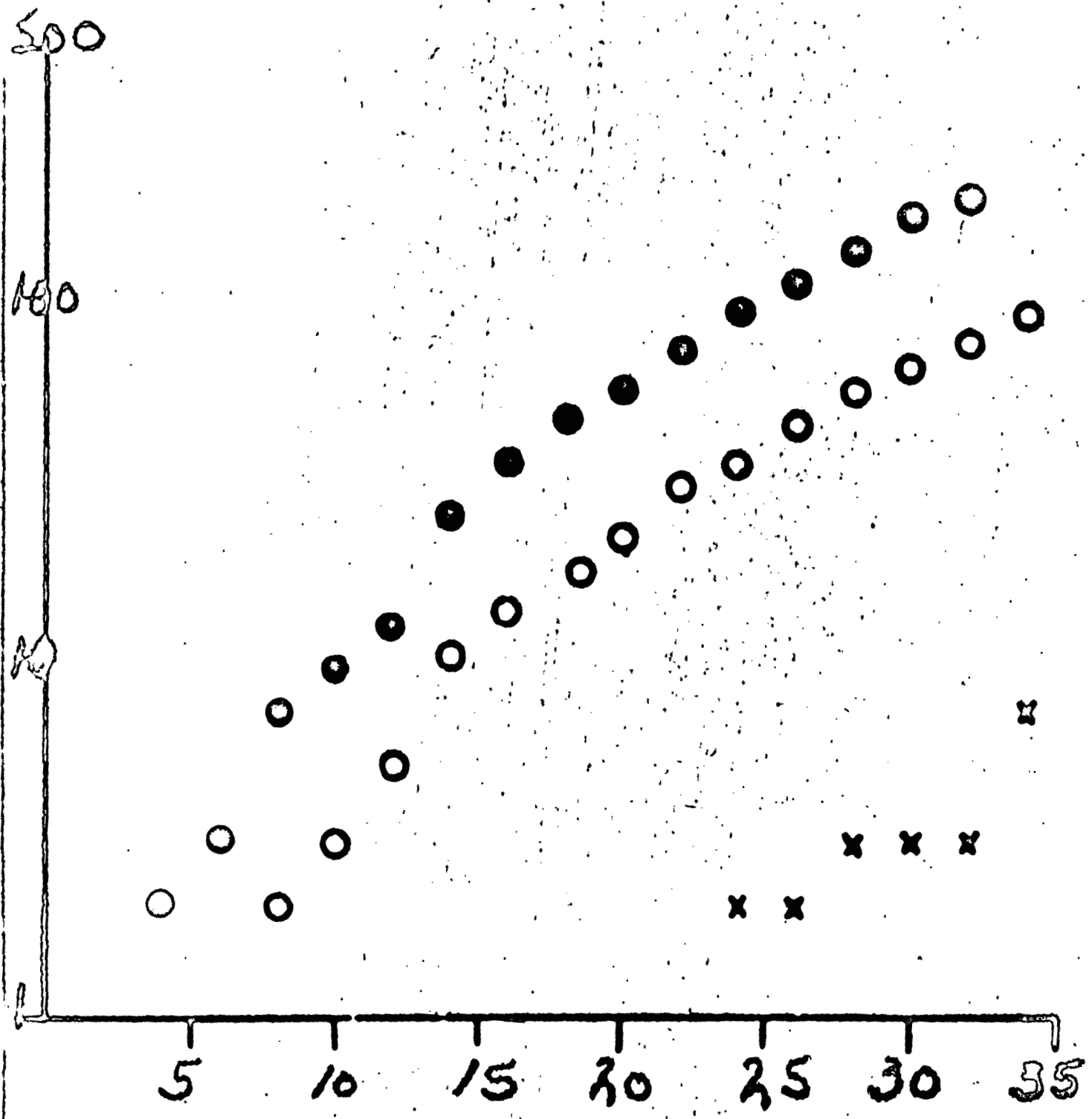


Fig. 15 The growth of problem No. 12, sixty generations.

○ total stolon points, ○ total hydranths, X red (generative) zooids.

