

*Sex-determination in Hydatina, with Some Remarks on
Parthenogenesis.*

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(Communicated by Adam Sedgwick, F.R.S. Received May 15,—
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[PLATE 11.]

Introductory.—Those writers who hold that the sex of an animal organism can be affected by a modification of external conditions during the period of development have of late years supported their case by reference to the parthenogenetic rotifer, *Hydatina senta*. Upon certain of the facts of reproduction there is a general consensus of opinion among those who have made a study of this species. Briefly summarised it is as follows:—

Three kinds of ova are produced by this rotifer, viz.: (1) parthenogenetic ova which develop into ♀'s; (2) parthenogenetic ova of smaller size which develop into ♂'s; and (3) fertilised eggs which always develop into ♀'s. Any given ♀ lays but one of these three classes of eggs during her lifetime. We may, therefore, distinguish three kinds of ♀'s by the eggs they lay, viz.: (a) ♀'s which produce ♀'s parthenogenetically, or *thelytokous* ♀'s; (b) ♀'s which produce ♂'s parthenogenetically, or *arrenotokous* ♀'s; and (c) the layers of fertilised eggs. Both thelytokous and arrenotokous ♀'s can be impregnated by the ♂. On the former impregnation has no effect. In the case of the arrenotokous ♀'s impregnation has no effect, unless it occurs during the first few hours after hatching. When this takes place such a ♀ produces fertilised eggs instead of the ♂ eggs which it would otherwise have laid. The layers of fertilised eggs are, therefore, arrenotokous ♀'s, which owe their special nature to the circumstance of having been impregnated by the ♂ during the earlier stages of their growth. And in this connection it is interesting to recall Lenssen's observation that the ♂ egg extrudes a single polar body, whilst the ♀ egg extrudes none.*

Though the thelytokous ♀'s cannot be fertilised they are able to give rise parthenogenetically to arrenotokous ♀'s as well as to other thelytokous ♀'s. The proportion of arrenotokous ♀'s so produced is subject to considerable variation, and to connect these variations with external conditions has been the object of those who have bred these animals with a view to throwing light upon the vexed problem of sex-determination.

* 'La Cellule,' vol. 14, 1898.

In a series of brief papers communicated to the French Institut, Maupas* held that temperature was the factor which determined the result, but that it produced an effect only in newly-hatched ♀'s before the eggs had commenced to develop in the ovary. To quote his words: "Au début de l'ovogenèse, au contraire, l'œuf est encore neutre et, en agissant convenablement, on peut à ce moment lui faire prendre à volonté l'un ou l'autre caractère sexuel. L'agent modificateur est la température. L'abaisse-t-on les jeunes œufs qui vont se former revêtent l'état de pondreuse d'œufs femelles; l'élève-t-on, au contraire, c'est l'état de pondreuse d'œufs males qui se développe."

More recently Nussbaum† has criticised Maupas' results. He has pointed out that under adverse nutritive conditions the eggs laid by a thelytokous ♀ are often no larger than those laid by an arrenotokous ♀ under normal conditions. Such adverse conditions Maupas must have brought about by raising the temperature, and Nussbaum suggests that Maupas mistook the small ♀ eggs then produced for ♂ eggs. Had he waited for them to hatch he would have realised their true nature. From my own experience, in so far as it goes, I am inclined to attribute some weight to this criticism of Nussbaum, and to agree with him in considering that an element of uncertainty is in this way introduced into Maupas' results.

Nussbaum supported his criticism by experiments and showed that a ♀ subjected to a high temperature before hatching or during the earlier period of its life may give rise to thelytokous ♀'s only (Experiments 21, 23, and 24, pp. 265 to 267).

Having rejected temperature as a factor in the determination of sex in *Hydatina*, Nussbaum turned his attention to other possible influences, and believed that he had found such a one in nutrition.

"Bei *Hydatina senta* bestimmt während einer gewissen Entwicklungsphase die Ernährung das Geschlecht des ganzen Geleges eines jeden jungfräulichen Weibchen. Wird das auskriechende Weibchen bis zur Reifung seines ersten Eies gut ernährt, so legt es nur weibliche Eier; wird es bis zur Geschlechtsreife mangelhaft ernährt, so legt es nur männliche Eier. Vor und nach dieser Periode hat die Ernährung keinen Einfluss" (p. 306).

In such a conclusion I cannot agree with Nussbaum, for reasons which will appear below. It is sufficient to note here that his own experiments do not always bear him out. Thus in Experiment 50, p. 283, he had a "Hungercolonie" on October 4, in which were many ♀ eggs. From the account it is evident that many of these must have hatched and have been

* 'Comptes Rendus,' 1890-91.

† 'Archiv f. mik. Anat.,' 1897.

without food during the supposed critical early stages before fresh *Euglena* was given them 25 hours later. Nevertheless, no ♂'s appeared in subsequent breeding from the culture.

From all this it must be apparent that neither modifications of temperature nor of nutrition afford a satisfactory explanation of the varying proportion of arrenotokous ♀'s which may occur in different cultures of *Hydatina*. There is, however, another explanation of this phenomenon which avoids the necessity of having recourse to external influences. It is possible that it may be the property of certain ♀'s to produce arrenotokous ♀'s in a definite ratio, and again the property of others to produce none. To test such a possibility it is obvious that the starting point for each generation must be the individual and not, as in all of Maupas' and many of Nussbaum's experiments, a collection of individuals.

Methods.—Breeding experiments were started in October, 1904, and, with short breaks, have been carried on until the present time. Each rotifer from which offspring were to be reared was isolated in that form of vessel known as a solid watch-glass, where it lived in about 2 c.c. of water containing *Euglena*. As the young hatched from its eggs they were similarly isolated by means of a fine pipette. If it was desired to test only their immediate offspring, some three or four of these were often placed together for the better economy of space and food. No ♀ was recorded as either thelytokous or arrenotokous until her eggs had been proved to hatch into either ♀'s or ♂'s. The average temperature of the room, as measured by a maximum and minimum thermometer, was about 16° to 18° C. On occasion it was lowered to 6° C. or raised to 24° C. *Hydatina* is extremely hardy and the chief difficulty in breeding it lies in the food supply, for it thrives far better on *Euglena* than on anything else. My supply of this Protozoon was derived from Mr. Thos. Bolton, of Birmingham, and was on the whole regular. In the occasional lean intervals particular experiments came to an enforced end, and my chief care was then to preserve certain strains from extinction. In one instance only was I compelled to have recourse for about 10 days to another food supply, and to make use of the bacteria and other small organisms which occur in a decoction of cabbage leaves. *Hydatina* can live on this diet, but does not thrive as it does upon *Euglena*. A further difficulty in connection with the food supply lies in its purification. The water in which *Euglena* lives usually contains numbers of *Hydatina* with their eggs. To remove these by a pipette is a tedious business, and there is always an element of uncertainty present in that a young rotifer or an egg may have been overlooked. Without doubt some of my earlier experiments are not absolutely trustworthy for this reason, and

I have consequently not made use of them in the following account. No such doubt, however, attaches to the later experiments, for the advent of a small centrifugal machine into the laboratory provided an easy and certain means of obtaining a culture of *Euglena* free of rotifers and their eggs.

Experimental Results.—My experiments have led me to the conclusion that among the rotifers I used were certainly three different types of thelytokous ♀'s, viz. :—

- A. ♀'s producing a high percentage of arrenotokous ♀'s.
- B. ♀'s producing a low percentage of arrenotokous ♀'s.
- C. Purely thelytokous ♀'s producing no arrenotokous ♀'s.

It will be convenient to consider the evidence for this conclusion under these three headings. Incidentally it may be mentioned that the production of ♂'s is, under laboratory conditions at any rate, quite independent of the season of the year.

Type A. ♀'s Producing a High Percentage of Arrenotokous ♀'s.

Only once was such a strain met with and bred over a series of generations. The result is shown in Fam. 2 (see diagram, Plate 11). It will be noticed that from the seventh generation onwards the proportion of arrenotokous ♀'s is high and bears to the thelytokous ♀'s a ratio of about 7 : 9, the actual numbers being 46 : 63. Here, as in other experiments, I was unable to trace any order in the sequence of the two types of ♀'s. The proportion of arrenotokous ♀'s among the earlier eggs of a family was neither higher nor lower than among the later ones.

Owing to an unfortunate failure in the food supply the strain was lost before experiments had been made to determine whether it was capable of giving rise to either type B or type C.

Type B. ♀'s Producing a Low Percentage of Arrenotokous ♀'s.

It has been my experience that if a ♀ is taken at random and bred from, it will usually turn out to belong to this class. As reference to Fam. 1 (see plate) shows, the percentage of arrenotokous ♀'s is low, but it is not possible to determine it with any degree of accuracy unless very much larger numbers are available. Even such figures may indicate but little, for they may represent merely the resultant of more than one definite ratio.

An interesting feature in this family is the production of an apparently pure thelytokous strain. Commencing with T* we have a series of 15 generations, comprising 177 individuals, in which no arrenotokous ♀ appeared. Failure of food then terminated the experiment, but I think

there can be little doubt of a purely thelytokous strain having arisen here. Though arrenotokous ♀'s are rare they yet average 2 to 5 per cent. in families of Type B, and the fact of their non-appearance among 177 individuals seems to be fairly conclusive evidence of the inability of the strain under consideration to produce them.

Type C. Pure Thelytokous ♀'s.

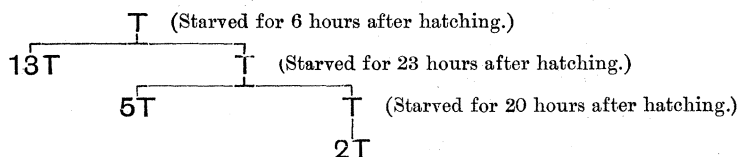
Rarer than Type B, but by no means difficult to obtain, are ♀'s which are incapable of giving rise to arrenotokous offspring. An example of such a strain is shown in Fam. 3, in which 55 generations including 178 individuals were bred and tested without the appearance of a single arrenotokous ♀. In another case 262 thelytokous individuals spread over 24 generations were produced, whilst in a third instance (Fam. 4 of plate) the strain was bred for 73 generations, during which 304 individuals were tested and shown to be purely thelytokous.

In view of these experiments I have referred to the ♀'s of Type C as purely thelytokous. By doing so I have no desire to ignore the possibility that such strains may eventually "break" and give rise to some arrenotokous ♀'s. Since, however, this has not occurred in my experiments the evidence for regarding them as I have done is very strong. In any case the nature of the ♀'s of Type C must be distinct from those of Types A and B.

Influence of Temperature and Nutrition.

It might be argued by some that the continuance of purely thelytokous strains was due to uniform conditions of temperature or of nutrition. Such, however, is not the case. In one of the experiments a purely thelytokous strain was subjected to considerable fluctuations of temperature. During a period of eight days it varied between 8° C. and 11° C., averaging 9° C. The rate of reproduction was much retarded, but in the many subsequent generations produced when the temperature was raised no arrenotokous ♀'s made their appearance. The same strain was afterwards subjected for four days to an average temperature of 22° C. (the extremes being 21° C. and 23° C.) without alteration of its purely thelytokous nature.

A number of starvation experiments were also made, of which the following may serve as an example. An egg from a purely thelytokous strain



(Fam. 3) was isolated soon after being laid and placed in clean water without a trace of *Euglena*. After hatching it was kept without food for six hours and then well fed. It laid 14 eggs which all became thelytokous ♀'s. One of these was again starved for 23 hours and, on being supplied with food, laid six eggs which all gave rise to thelytokous ♀'s. Lastly, one of these six was starved for 20 hours. Though well supplied with food at the end of that time it experienced some difficulty in growing up and laid only two eggs before it died. From both these eggs hatched out thelytokous ♀'s. The relation of these generations is shown in the accompanying scheme. In none of the other similar experiments made with purely thelytokous strains did any arrenotokous ♀'s appear, neither was the proportion of ♂'s raised as the result of starving families of Type B.

Reference was made above to an instance in which, owing to failure in the *Euglena* supply, a decoction of cabbage was used as food. This happened during the 54th and 55th generations of a strain of Type C (see Fam. 4). The animals were poorly nourished and laid very few eggs. Nevertheless the strain just managed to tide over the period of dearth, and on *Euglena* being again forthcoming it went on for 17 further generations before the experiment was stopped. During this time 134 individuals produced were proved to be thelytokous and none were found to be arrenotokous.

In the face of such facts as these it is difficult to entertain the opinion that either temperature or nutrition have any influence in determining the production of arrenotokous ♀'s.

The varying proportion of arrenotokous ♀'s in different cultures of *Hydatina* apparently depends upon the existence of ♀'s of different zygotic constitution, and the solution of the sex problem seems to lie in the determination of the unit-characters involved. The data for such a solution are at present inadequate. Before it is possible to frame a theory we must have further knowledge on the following points:—

(a) The number of different types of ♀ that exist as judged by the criterion of their relative output of thelytokous and arrenotokous ♀'s, and the relation of these types to one another.

(b) The possible existence of zygotically distinct types of ♂'s and the types of ♀'s from which they respectively arise.

With regard to the former point we have evidence for the existence of (1) purely thelytokous ♀'s, (2) of ♀'s producing arrenotokous ♀'s in the proportion of about 7 in 16, (3) of strains of ♀'s which produce but few arrenotokous ♀'s and in which the rather irregular numbers may be due to the co-existence of two types at present indistinguishable owing to imper-

fection of analysis, and (4) of ♀'s which, according to Maupas,* produce three arrenotokous ♀'s out of every four. Beyond the fact that (1) may arise from (3) we know nothing of the inter-relations of these various types.

Whether or not there are zygotically distinct types of ♂'s we are at present quite ignorant. The possibility might, however, be tested by a system of fertilisation experiments and subsequent breeding from the ♀'s hatched.

The Nature of Parthenogenesis.—Any interpretation of the facts which is based upon inherent differences in the zygotic constitution of individual ♀'s must necessarily lead us to modify our views as to the nature of parthenogenesis. A given ♀ can be constructed on but a single zygotic plan. Consequently if she produces eggs without the formation of polar bodies, as Lennsen† has suggested, these eggs, provided they contain the somatic number of chromosomes, ought all to possess the same zygotic constitution and the same zygotic properties. But if we assume, and the assumption seems warranted by facts, that external influences are without effect, we must then infer that a ♀ which produces both arrenotokous and thelytokous ♀'s is producing two entirely different kinds of egg. In the light of recent Mendelian research such a condition implies a process of segregation which is probably bound up with a reduction in the somatic number of chromosomes, and the subsequent restoration of that number by the union of two gametic cells each containing the reduced number. Though without evidence either way we have no reason to suppose that the somatic number of chromosomes varies in *Hydatina* during successive generations. We are, therefore, led to suppose that, somewhere antecedent to the formation of an ovum, there is a gametogenic process, and that the recombinations which take place among the gametes so formed determine the proportion of arrenotokous ♀'s. In other words, it seems not inconceivable that the thelytokous ♀'s are really hermaphrodite, though the ♂ gametes may not exhibit the orthodox form of spermatozoa.‡ Such a view would account for the observed absence of polar bodies in the ♀ eggs, for it must be supposed that the process of reduction and fertilisation takes place before the accumulation of yolky material. The ♀ eggs would on this view be comparable, not to the parthenogenetic ♂ eggs, but to ♂ eggs which had undergone fertilisation with the formation of "winter" eggs.

On the other hand it must not be forgotten that, although unable to

* 'Comptes Rendus,' October 6, 1890.

† 'La Cellule,' vol. 14, 1898.

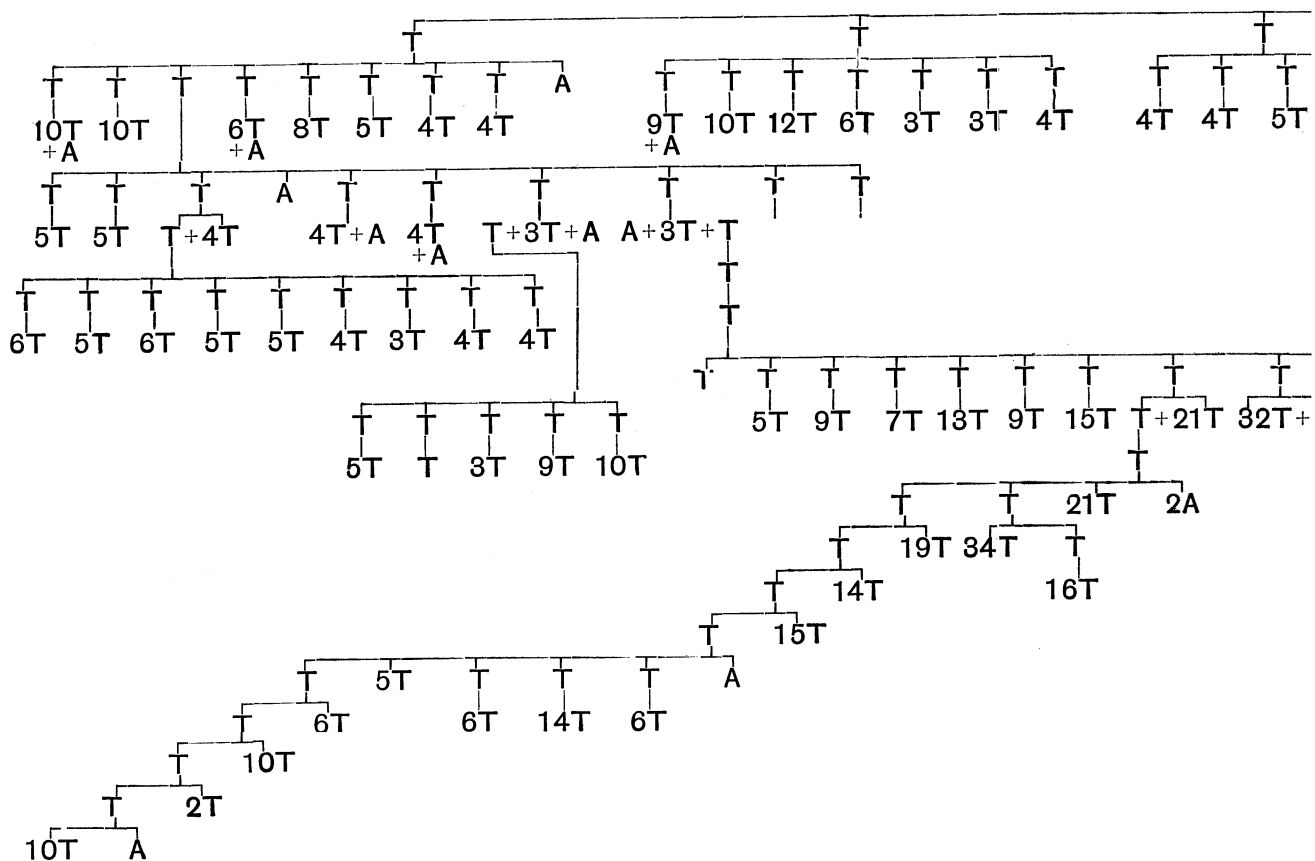
‡ In this connection may be mentioned the interesting discovery of Brauer, that in the parthenogenetic egg of *Artemia* a second polar body is extruded, but immediately re-enters the ovum and fuses with the ♀ pronucleus.

observe the extrusion of a polar body, Lennsen* nevertheless describes the beginnings of karyokinesis in the parthenogenetic ♀ eggs. The process, however, appears to be arrested half way, and no segregation of chromatic material seems to occur. Whether there is such segregation must be left for future investigation to decide. The problem can be decided only by tracing accurately the fate of the chromosomes, and it is with a view to stimulating further work on the part of the histologist that I have put forward the highly speculative suggestion outlined above.

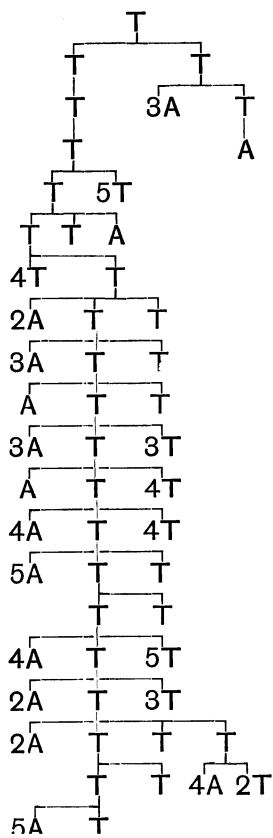
Before concluding these remarks on the nature of parthenogenesis a few words may be devoted to another aspect of the problem. Since the publication of Maupas' experiments on ciliated infusorians it has been generally held among biologists that the effect of conjugation is to arrest a process of senile decay otherwise inevitable in animal stocks which exhibit the phenomenon of asexual reproduction. In other words, there must be a limit to the amount of protoplasm produced in the period between two successive acts of conjugation. Among lowly organisms such as the protozoa this amount may be very large. Among the more highly differentiated metazoa it is relatively small, and where reproduction is entirely sexual it is limited to the amount produced during the lifetime of an individual. A pure thelytokous strain of *Hydatina senta* offers a remarkable contrast to the rest of the metazoa. If we reckon the volume of an individual at 0.01 cubic millimetre, and suppose that each is capable of producing 30 eggs it is a matter of simple calculation to show that the volume of protoplasm producible in 65 generations (*i.e.*, in less than a year) would form a solid sphere of which the dimensions would be such that it could not be contained within the limits of the known universe.† We are, therefore, driven to one of two alternatives—either that what may be termed the potential productivity of protoplasm is immeasurably greater in *Hydatina* than in all other metazoa, or that some process of conjugation occurs at one stage or another in the so-called parthenogenetic reproduction of this form. For reasons already stated I am inclined to favour the latter view, and it is in the hope that the matter may attract the attention of the histologist that I have ventured upon the calculation which forms the substance of this last paragraph.

* *Loc. cit.*, p. 437.

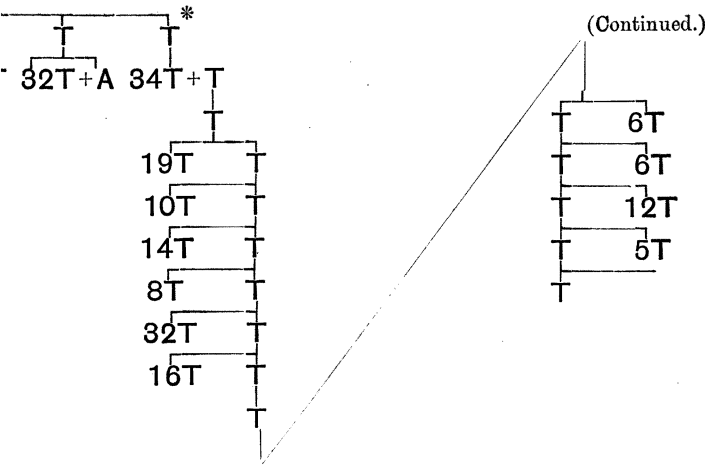
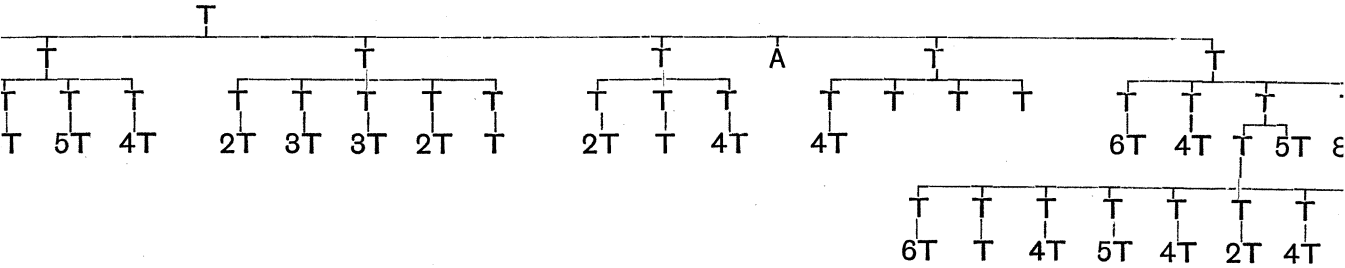
† For assistance in making the calculations upon which this statement is based I am greatly indebted to my astronomical friend, Mr. Cookson, of Trinity College, Cambridge.



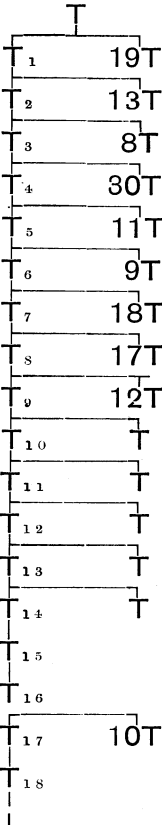
FAMILY 2.



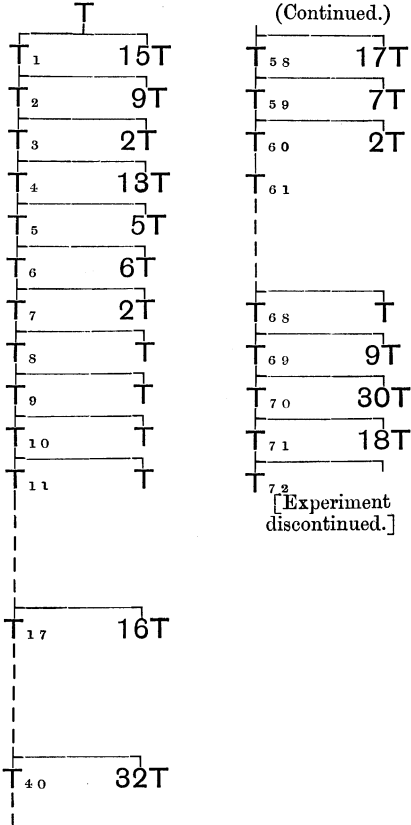
FAMILY 1.

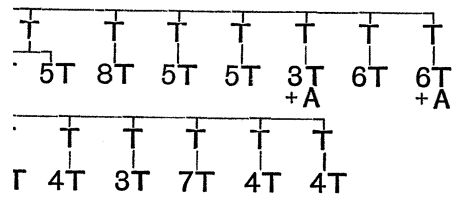


FAMILY 3.



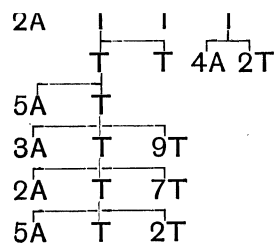
FAMILY 4.





1.)
7T
7T
2T

7
7T
0T
8T
nt
1.]



17 101
 T 18
 &c.
 T 40 25T
 &c.
 T 55
 [Experiment
 discontinued.]

T 40 32T
 T 54 } Fed on a
 T 55 } decoction
 of cabbage.
 T 56 24T
 T 57 19T
 (Continued
 above.)

EXPLANATION OF DIAGRAM, PLATE 11.

On the plate are given details of four families of parthenogenetic ♀'s. Fam. 1 contains a few arrenotokous ♀'s, Fam. 2 contains many, whilst Fam. 3 and Fam. 4 show none (*cf.* Types B, A, and C, pp. 226, 227). T denotes a ♀ proved to be thelytokous. A denotes a ♀ proved to be arrenotokous. The small figures in Families 3 and 4 signify the number of the generation from the original parent of the strain. Thus in Family 3, T₉ + 12T means that in the ninth generation 13 ♀'s from the same parent (T₈) were proved to be thelytokous, and that one of these was used as the mother of the tenth generation—the rest being discarded. Where apparent gaps occur, *e.g.*, in Fam. 3, between T₁₇ and T₄₀, it means that a single ♀ was used in each generation to continue the strain, but that the sisters of these individuals were not bred from.

On the Julianiaceæ, a New Natural Order of Plants.

By W. BOTTING HEMSLEY, F.R.S., F.L.S., Keeper of the Herbarium and Library, Royal Botanic Gardens, Kew.

(Received June 6,—Read June 28, 1906.)

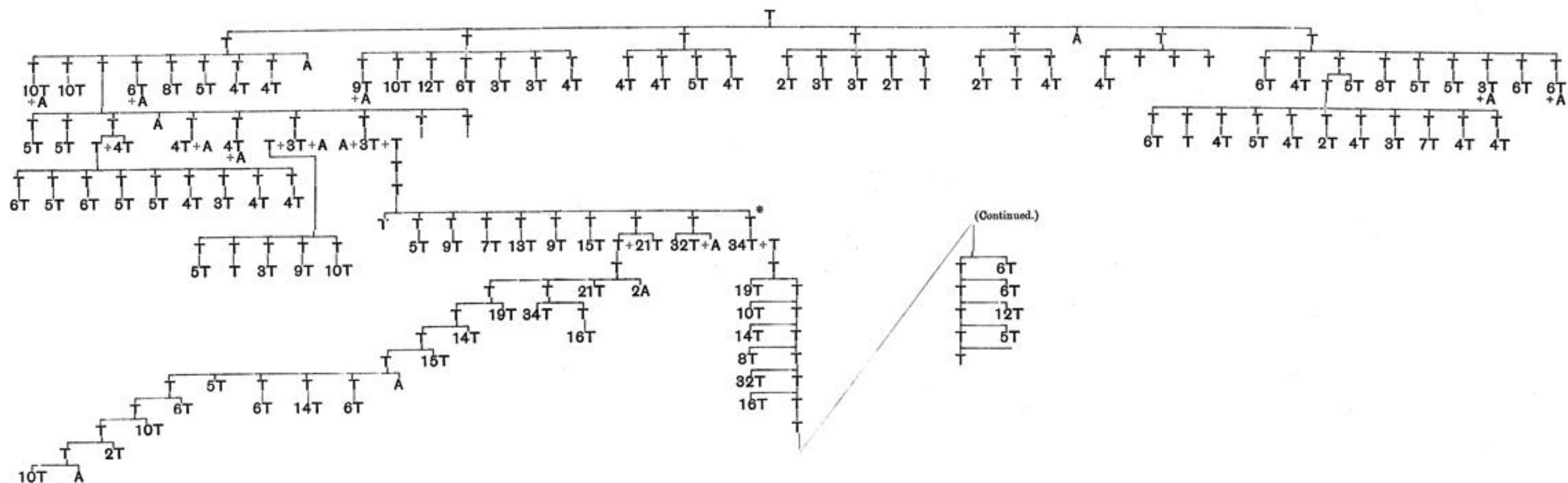
(Abstract.)

I.—GENERAL DESCRIPTION.

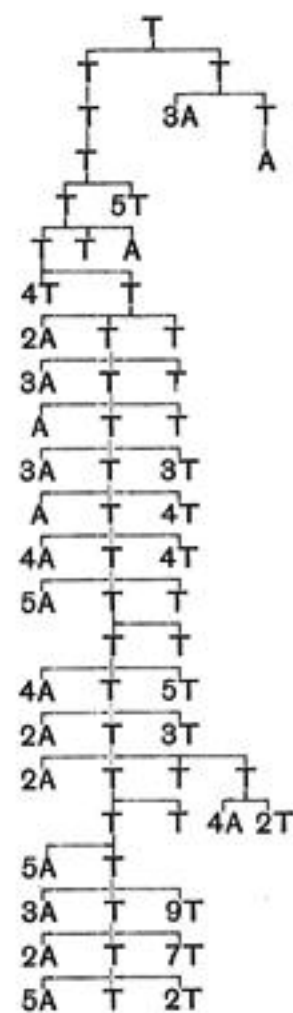
The Julianiaceæ comprise, so far as at present known, two genera and five species. They are resiniferous, tortuously branched, deciduous, diœcious shrubs or small trees, having alternate, exstipulate, imparipinnate leaves, from about one to three decimetres long, clustered at the tips of the flowering branches and scattered along the short barren shoots. The flowers are small, green or yellow-green, quite inconspicuous, and the males are very different from the females. The male inflorescence is a more or less densely branched axillary panicle or compound catkin, from 2½ to 15 cm. long, with weak, thread-like, hairy branches and pedicels. The male flowers are numerous, 3 to 5 mm. in diameter and consist of a simple, very thin perianth, divided nearly to the base into four to nine narrow equal segments, and an equal number of stamens alternating with the segments. In structure and appearance they are almost exactly like those of the common oak. The female inflorescence is similar in structure to that of the sweet chestnut, consisting of an almost closed, usually five-toothed involucre, borne on a flattened pedicel and containing three or four collateral flowers, of which the two outside ones are, perhaps, always abortive.

At the flowering stage, the female inflorescences, including the narrow

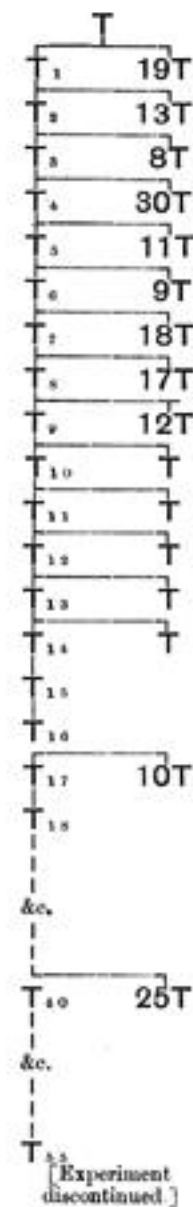
FAMILY 1.



FAMILY 2.



FAMILY 3.



FAMILY 4.

