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Researches in the Genus *Taraxacum*

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RESEARCHES IN THE GENUS TARAXACUM

by

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RESEARCHES IN THE GENUS TARAXACUM

Introductory

These researches were undertaken with a view to working out certain little known phases of the life history of our local species of dandelion, and particularly with a view to confirming or disproving the following widely accepted conjecture of Raunkjiaer;-

"Species Danicae Taraxaci castratione agamice propagari demonstratum est; species omnes Taraxaci semper parthenogenetice propagari verisimile est."

The problem has been found to involve difficulties ~~a~~ and obscurities which will require considerable time to effectually disentangle, but results have already been secured which we believe ourselves justified in setting forth.

During the work we have been much in debt to the late Dr. Charles E. Bessey for his cheerfully given and suggestive help, as well as to his associates and to Dr. Florence McCormick for kindly aid.

A Brief Historical Resume of the Problem.

Schwere, working on Taraxacum officinale Web. in 1896, traced the development of the embryo from the egg nucleus. He found the egg-apparatus ready in specimens prepared "lange vor der Anthese", but looked in vain for a stage showing union of sex-cells, although figuring what he took to be the tip of a pollen-tube.

He quotes Hildebrand (1869) as saying that seed-production is impossible in Taraxacum material protected from insects, and asserts that border flowerets - the first to open - are barren oftener than the later opening disc flowerets, in default of actually witnessing fertilization.

The comments of Müller, in his work on "The Fertilization of Flowers", may be of more than passing interest. Granting that Taraxacum officinale L. attracts numerous insect visitors during sunny weather, he deduces that it has either retained, or again acquired, the possibility of self-fertilization, since its earliest and latest blossoms are likely to be left unvisited.

In 1900 Andersson and Hesselman suspected parthenogenesis in Taraxacum phymatocarpum Vahl, found at Spitzbergen, basing their hypothesis on its lack of pollen.

In 1903 Raunkjiaer performed his classical castration experiments, removing anthers, styles, and stigmas by simply cutting across the flower head. In spite of this presumably effective prevention of normal fertilization, viable seeds were produced on

a) the pollenless forms, T. paludosum Scop. (T. balticum Dahlst.), T. ostenfeldii Raunk., T. speciosum Raunk., T. decipiens, Raunk. (also T. croceum Dahlst. and T. phymatocarpum Vahl?)

b) the hermaphroditic forms, T. vulgare Lam., T. intermedium Raunk., T. gelertii Raunk., T. obovatum (Willd.) DC, and T. glaucanthum Led., (also T. erythrospermum Andrz. or T. laetum Dahlst.?).

In the pollen-bearing forms Raunkiaer was unable to find trace of germinating pollen-grains. Relying on Schwere's proof of the egg-origin of the embryo in T. officinale Web., Raunkiaer predicates the existence of true parthenogenesis in the forms he investigated, and ventures the speculation, quoted in our introductory paragraphs, that probably all forms of the genus are parthenogenetic.

Murbeck, in 1904, followed up these experiments by studying the morphological changes involved. He compared the formation of embryos in normal and castrated plants of T. vulgare (Lam.) (T. vulgare (Web.) Wigg.?) Raunk., a pollen-producing form, and T. speciosum Raunk., which is pollenless. Castration he found to be without effect on embryo production, and in both species he found a fairly typical embryo sac development, with the embryo arising from the egg-cell without apparent special stimulus.

The egg-apparatus he described as consisting of three transitory antipodals, two large endosperm nucleii which soon fuse and redivide, two elongate purse-shaped synergids with vacuoles in their free ends, and an egg-cell distinguished by a very large nucleus lying near the cell tip. The egg, he found, elongates to mid-sac and develops a fine cellulose wall before nuclear fission occurs. On uncastrated plants of T. vulgare with plenty of pollen Murbeck was unable to find pollen tubes, while in November T. speciosum, pollenless, showed young embryos in unopened flowers.

Stimulated by these various contributions Juel in 1904 issued a preliminary report of work on "Die Tetradenteilung in der Samenanlage von Taraxacum". His work was done on what he calls T. officinale, and he describes the seed anlagen as having from the first a normal structure. The megaspore mother cell passes through synapsis and diakenese only, however, resulting in the formation of a large basal and small apical daughter cell, the former developing into the embryo sac. Juel confirms the history of embryo development as traced by Schwere for the same(?) species. Four counts at diakenese showed between twenty and thirty chromosomes, and comparable numbers were obtained in vegetative tissue counts. "Ich folge . . . dass bei Taraxacum wahrscheinlich keine Chromosomenreduktion stattfindet. . . Die Tetradenteilung ist also bei Taraxacum eine scheinbare und eine unvollstandig".

Writing in 1905 on parthenogenesis among the flowering plants, Kirschner says of Taraxacum that in the open as a rule the stigmas were found richly covered with pollen, but that the pollen grains could neither be brought to germinate artificially, nor was their germination observed on the stigmas. He also found in his study of numerous Taraxacum stigmas that foreign pollen grains were germinating on them, but never pollen grains of their own species, abundant as the latter might be.

Dahlstedt, writing in 1907, speaks as follows, after briefly reviewing the work of Raunkiaer, Juel, and Murbeck. "Die von diesen Forschern untersuchten Arten gehören verschiedenen und zuweilen einander sehr fern stehenden Gruppen an. Da also die Apogamie (Parthenogenese) nicht nur eine weite Verbreitung innerhalb verschiedener Verwandschaft Gruppen der Gattung hat, sondern auch bei Arten von vielen getrennten Florengebieten vorkommt, ist anzunehmen, dass sie zweifellos von sehr hohem Alter ist. Aus den bisher gemachten Untersuchungen ist jedoch nicht endgültig festgestellt dass sich alle Sippen der Gattung apogam verhalten, obwohl dies nach den angefuhrten Tatsachen zu urteilen betreffs der Mehrzahl wahrscheinlich ist. Wenigstens deuten einige meiner Beobachtungen darauf hin, dass bei einzelnen Sippen Samenanlagen vorkommen, die mit Eizellen versehen sind, bei welchen für ihre weitere Entwicklung möglicherweise eine Befruchtung nötig ist."

Continuing, Dahlstedt tells of finding numbers of sterile seeds in fertile heads of certain species groups, and while admitting that it may have been due to causes other than failure of pollination, he intimates that the subject is worthy of cytological work. He likewise expresses a belief, based on the frequent retention of mechanisms rendering crossing feasible, "dass noch Arten vorkommen können, bei denen die geschlechtliche Vermehrung noch nicht ganz verloren gegangen ist, dass aber bei sonst apogamen Arten einzelne Samenanlagen mit normal angelegten Eizellen versehen sein können."

The same year, 1907, witnessed the publication of Handel-Mazetti's "Monographie der Gattung Taraxacum, from the "biological" section of which we take the following translation. "As firmly . . as the lack of fertilization seems to be established by the investigations of Murbeck, I am nevertheless obliged to think necessary the continuance of analagous investigations in comprehensive measure under the widest possible variations of environment and climate, before the existence of bastards in the genus <u>Taraxacum</u> can be indicated as absolutely exclusive".

Rosenberg, writing in 1909, gave a valuable resume of the facts known about apogamy, adding to them the result of his own work, showing that T confertum, at least, presents typical reduction in the megaspore from 16 chromosomes to the haploid number of 8, but confessed ignorance as to whether or not such egg was "befruchtungsbedürftig". He found that Taraxacum obeyed the rule of a diploid chromosome count in apogamous forms equal to about twice the normal diploid count in sexual species, but seems unable to explain this.

Ikeno, writing in 1910, tells that E. Tanaka, working in Tokio during the previous year had found T. albidum fruiting abundantly in spite of castration, while T. platycarpum would not. Ikeno himself found what he describes as "wild mutants" of the latter form growing in the Tokio Botanical Gardens, none of which "mutants" were parthenogenetic, or even self-fertilizing , while he confirmed the results of Tanaka with the other species. When artificially self-fertilized, T. platycarpum, the sexual form, produced only 8% viable seeds, ripening all the remainder as empty fruits. Ikeno conceives on this basis that Taraxacum, like Hieracum, has some parthenogenetic and some sexual forms.

We take from a paper by Schorbatow, 1913, entitled "Studien an Taraxacum officinale Wigg." only the following points as bearing on the work in hand.

The castration of the blossoms never influences the viability of the seeds produced unfavorably.
In diakenese one seldom sees an arrangement of chromosomes indicating heterotypic division - such cases can only be of an atavistic nature. He mentions also as remarkable the occurence of amitotic nuclear division in the embryo sac.

Material

The flora of Nebraska is commonly (Petersen, Britton's Manual) said to contain two species of *Taraxacum*, *T. vulgare* (Lam.) Schrk., given as *Leontodon taraxacum* L. in the new second edition of Britton & Brown's Illustrated Flora, and *T. laevigatum*, (Willd.) DC , listed as *L. erythrospermum* (Andrz.) Britton by said authors.

Since the *Taraxaca* of the world have been recently (1907) monographed by Handel-Mazetti, who was cognizant of the species of Britton & Brown in a cosmopolitan way, I feel constrained to accept his nomenclature, and have done so above. It is necessary to observe, however , that *T. laevigatum* shows variations in length of achene beak from the descriptions of both Handel-Mazetti and Britton & Brown.

Both species produce pollen in abundance. Dr Ernst Bessey finds that *T. laevigatum* blooms only in spring and fall, while of course *T. vulgare* blossoms at any time the weather permits. Although *T. laevigatum* tends to remain smaller than the other species, there is no safe criterion for distinguishing the two forms save the color of the ripened achene coat, which is red in *T. laevigatum* and grey in *T. vulgare*. I have found, however, that there

there is a rather constant difference in the form of the embryo sac.

_T._ _vulgare_ is the same form mentioned as such in the historical resume just given, doubtless, while it is not improbable that _T._ _laevigatum_, under another name, was among the number experimented upon by Raunkjaer.

Methods

Our method of attacking the problem has been two-fold, experimental and morphological.

<u>Experimental method</u>. Raunkjaer's castration experiments were repeated with both species as follows,

The whole flower head of an unopened bud was severed with a sharp razer just above the ovaries, removing styles, stigmas, and all pollen grains. The plant was then isolated and protected from pollination from any source. These experiments were tried on buds of various ages, never older than a day previous to opening, and under natural and greenhouse conditions.

<u>Morphological method</u>. By means of sections the life history of the female gametophyte of both species was sought in an endeavor to ascertain if the production of the embryo sac was normal, and whether any nuclear fusions could be observed or not.

Experiment with various chromacetic killing fluids, Flemming's stronger and weaker, and acetic alcohol resulted in the exclusive use of the last named agent, which permitted the delicate structure of the embryo sac to be fixed without plasmolysis, by virtue of its almost instantaneous penetration. Single flowers were killed in numbers, but on the whole it was found most satisfactory to cut the flower heads into blocks with a base two to three millimetres square for killing.

The material was sectioned in paraffin, 12-14 micros microns being found the proper thickness to show most stages advantageously. A little material was stained in iron-alum haematoxylin, but Flemming's triple stain was given the preference because of its ease of working and much greater transparency.

Results

<u>Experimental</u>. Save in a couple of cases where the severe wound caused by castration of the flower head had become infected resulting in rot, the castrated plants produced seed normal in every respect save that the beak was frequently shortened. Both species, without visible distinction, produced such normal seed.

Furthermore, seed so produced germinated and produced healthy plants, some of which are now being held to test their seed character.

<u>Morphological</u>. As noted in our historical resume the life history of the female gametophyte of <u>T. vulgare</u> has been more or less worked over by Murbeck, Juel and others, hence our main concern was to discover the processes occurring in <u>T. laevigatum</u>, comparing them with what I found to be the case in <u>T. vulgare</u>, and finally comparing both sets of results with what previous work was available.

The megaspore mother cell was found in both forms to divide into two daughter cells, instead of the usual four, which two daughter cells showed distinct development of a separating wall. In <u>T. laevigatum</u> the megaspore mother cell was remarkable in showing, as late as the prophase of the first mitosis, remnants of the parietal cells much crushed and flattened, but still adhering to its tip. In fact one of these parietal cells was still discernible in the two-megaspore stage, as figured.

In both forms there is present the embryo sac development of the second type of Schniewind-Thies, namely the development of two potential megaspores, only one of which functions to form the embryo sac, the other breaking down.

In _T._ _laevigatum_, all cases so far discovered show that the upper or chalazal one of the pair of megaspores breaks down, leaving the micropylar or lower one to function. This is the exact reverse of what I found to be the case, with the other species, and what Juel describes for _T._ _officinale_ (_vulgare_?). It would be manifestly unsafe in the present stage of our work to say that these appearances represent consistent differences between the two species. They may hold with fair constancy, but certainly not without frequent exception, for Schniewind-Thies has shown free variations within a single species in this matter

The remaining megaspore, be it upper or lower, rapidly divides, having apparently lost its wall at the breaking down of its sister cell. Unfortunately I have been unable thus far to obtain mitotic figures at this stage, which will be invaluable in the ultimate solution of the problem.

The two nucleii so produced migrate at once to opposite ends of the embryo sac, where each undergoes two successive divisions.

The remaining stages are quite generalized. The four nucleii at the upper end of the sac organize the three antipodals, while the four at the lower or micropylar end organize the egg apparatus, consisting of the egg and two synergids. This leaves a free polar nucleus at each end, and these two nucleii next migrate to the middle of the sac and fuse, as figured, to form the primary endosperm nucleus.

The three cells of the egg apparatus seem typical in every way, the egg being marked by its large terminal nucleus and basal vacuole, while the synergids show characteristic terminal vacuoles and basal nucleii.

The embryo, so far as observed, develops from the egg, both synergid nucleii and endosperm nucleus retaining their integrity for a time at least.

The figures show clearly the peculiar narrowness of the embryo sac in T. laevigatum, as contrasted with the more rotund form found in the other species. As far as observed this difference seems to be constant, although with the development of the embryo the narrower sac naturally widens out more or less.

The microspores or pollen grains were observed in all stages from that of the mother cell to maturity, but all attempts to detect the formation of a pollen tube, or discharge of pollen nucleii were futile. The stigmatic surfaces of the dandelion are closely folded together until the flower opens, and the anthers which surround the style can only be opened at the time of blooming by the tearing action of the vertical barbs with which the style is equipped, when with the unfolding of the flower the style shoots up rapidly.

No opened flowers were found which did not show embryo development pretty well advanced. Furthermore, flowers taken a day or two before opening consistently showed embryo development already begun.

Finally, no certain trace of any pollen tube was found in micropyle or base of embryo sac, but this has no value as evidence, obviously.

Conclusions and Summary

The finding of embryos in unopened flowers, and the success of the so-called "castration" experiments cannot, singly or together, prove the existence of parthenogenesis. These conditions simply limit the possibilities as follows, to,

a) self-pollination while the flower is closed, and externally quite immature - cleistogamy.

b) embryo production, with or without previous nuclear fusion, from cells of the nucellus (sporophytic tissue).

c) embryo production, with or without nuclear fusion, fr from some non-sexual nucleus of the female gametophyte.

d) embryo production, with or without nuclear fusion, from the egg.

In eliminating the first possibility we can depend as yet on nothing stronger than reasonable negative evidence. Closed flowers, showing the beginnings of embryo formation, had every pollen grain intact, with not the slightest evidence of tube formation or nuclear transfer. Our basis for this statement is the examination f of serial sections complete for the flöwer. Neither is it likely, although not impossible, that pollen tubes would be formed early enough to escape injury and destruction from at least a good share of the castration experiments.

The second and third possibilities can likewise be even more summarily disposed of, at least as normal occurences, by our finding, which agrees with that of t other workers previously mentioned, that the embryo arises from the egg itself, and not from nucellar tissue, endosperm or synergid nucleii.

Since the integrity of the various nucleii composing the female gametophyte has been traced beyond embryo beginning, if any nucleus (aside from the male nucleus, which has been eliminated with good show of probability) fuses with the egg previous to embryo formation, it must be a nucleus from the surrounding sporophytic tissue.

The observations so far made give no warrant for the thinking this alternative probable, but do point decidedly to true parthenogenesis, with the embryo arising from the unfertilized and unstimulated egg. However, complete certainty regarding this probable state of affairs must wait until successful chromosome counts are secured .

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Explanation of Plates

All figures were drawn through Leitz #8 eye piece and #6 objective. Figs. 3 and 11 were drawn with the aid of a Spencer camera lucida, while the remainder were drawn by means of a Leitz drawing apparatus, for which approximately two-thirds must be added to the normal magnification of the lenses used.

Unless otherwise stated, all figures represent stages in the life history of _T. laevigatum_.

Fig. 1. Megaspore mother cell just previous to division.

Fig. 2. The two megaspores, with parietal cell still clinging to the micropylar megaspore.

Fig. 3. Breaking down of micropylar megaspore in _T. vulgare_.

Fig. 4. Breaking down of chalazal megaspore, and first division of functional megaspore.

Fig. 5. Migration after first division of megaspore.

Fig. 6. Four-nucleolate stage (amitotic?) of embryo sac.

Fig. 7. Eight-nucleate stage of embryo sac, showing approach of polar nucleii.

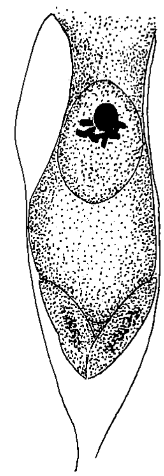
Fig. 8. Fusion of the polar nucleii to form the primary endosperm nucleus.

Fig. 9. Mature embryo sac, showing three antipodals above, endosperm nucleus in centre, egg with terminal nucleus, and two synergids, one with nucleus showing dimly.

Fig. 10. Mature embryo sac of T. vulgare, showing greater width than the other species. The antipodals are partially broken down.

Fig. 11. Early embryo, showing the synergid nucleii still in place

Fig. 12. Early embryo of T. vulgare, showing the synergids and endosperm daughter nucleii in place.



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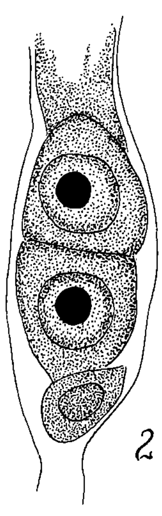

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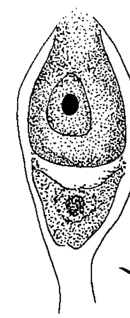

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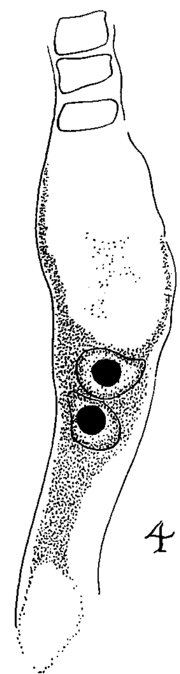

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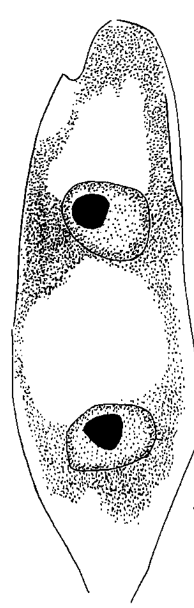

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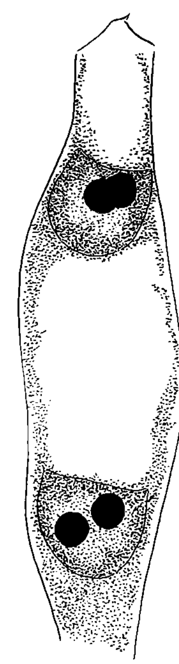

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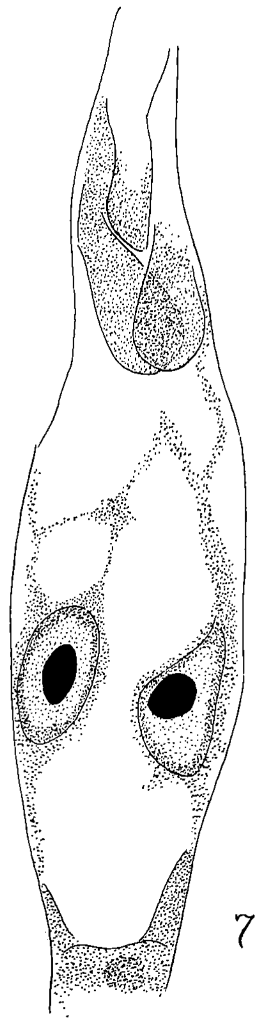

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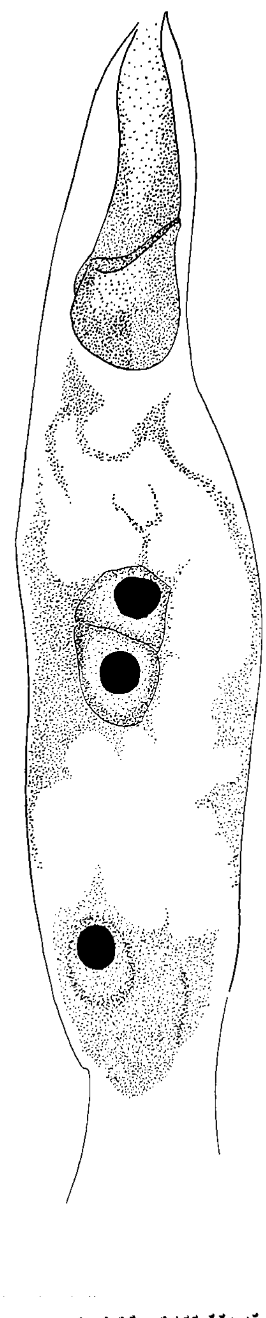

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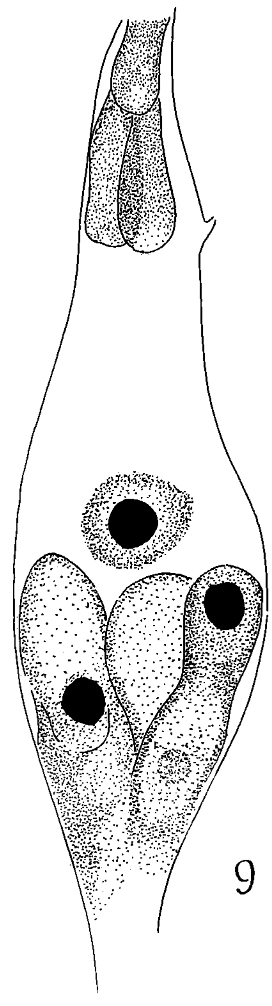

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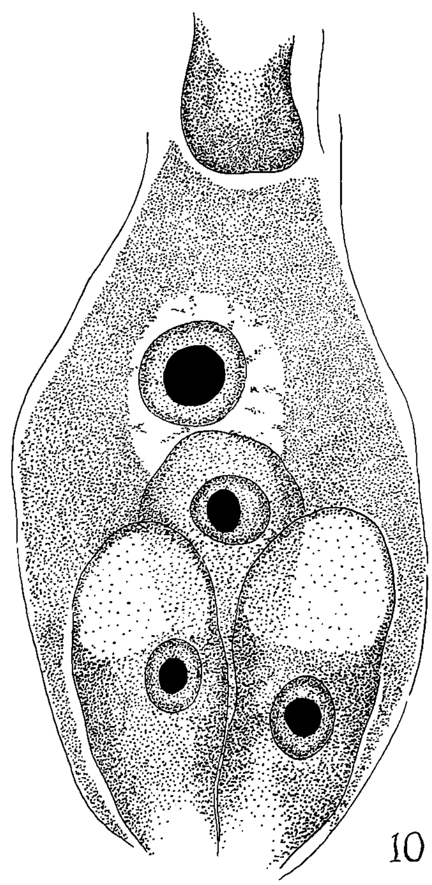

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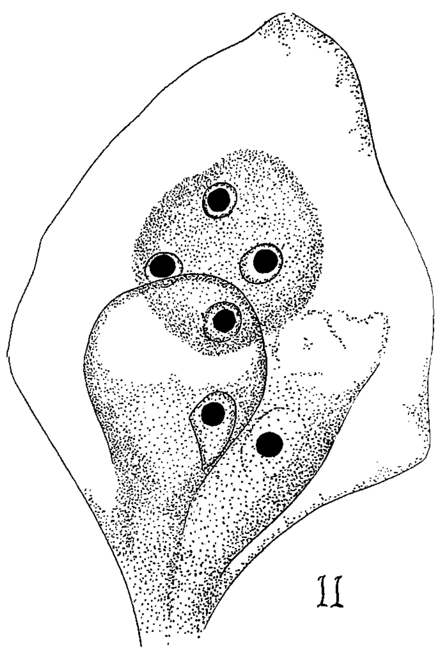

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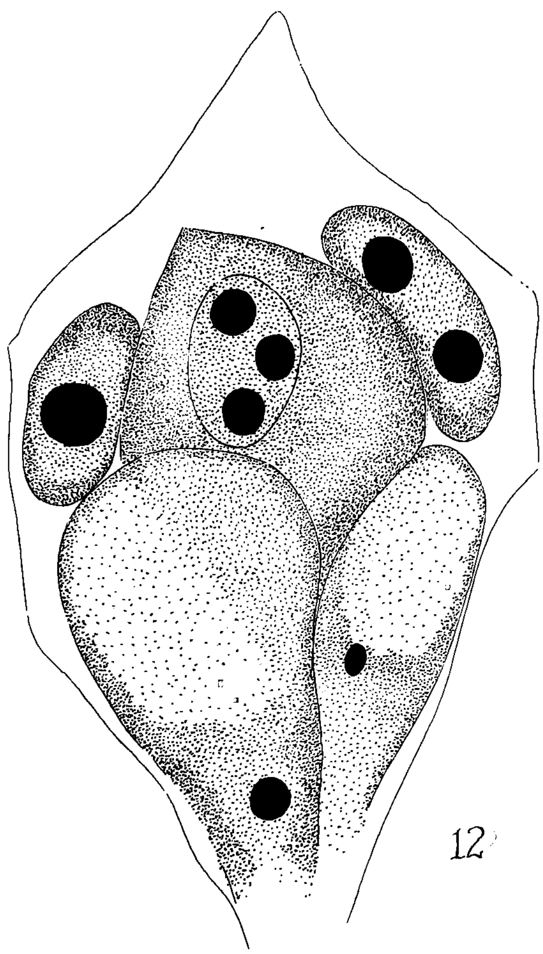

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