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Development of pollen in the Pinaceae and conclusions; a translation of Wilhelm Hofmeister's 1848c paper "Ueber die Entwicklung des pollens"

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Abstract

Hofmeister (1824–1877) confirmed that the nuclear and chromosomal features of cells seen in *Tradescantia* extended to distantly related conifers. This was despite the very different anatomy of anthers and megasporophylls and showed that nuclear and chromosomal phenomena were basic processes in biology.

These papers were written before the scientific format for papers was standardized and so introductory material, methods and conclusions were mixed together in the form of a stream of ideas. Hofmeister tended to end papers after presenting little more than raw data with clarifying notes. This may be because theorizing was reserved for larger works, such as his work on alternation of generation (Hofmeister 1851), or for lecture presentations. However, the great value of papers like this is precisely the bias in favor of raw data descriptions because this allows us to see exactly how the theories with which we are familiar have been formulated. The detailed study of botanical history is certainly illuminating and valuable for scientists who wish to know where current ideas originated.

Introduction

Eduard Adolf Strasburger (1844–1912) was an active scientist in the last decades of the 19th century and cited Hofmeister's early contribution to plant cell biology (Strasburger 1895). In all three of these papers (Witty 2015a, b and this paper) Hofmeister mentioned phenomena that were significant to Strasburger's later work on formation of the cell plate preceding cell division. The cell plate was a concept invented by Strasburger in 1876 as the concept we accept today (reviewed in Timberlake 1900) and which was anticipated by the work of Hofmeister. In these papers the cell plate was clearly observed in unstained cells, probably because of the combined refraction from vesicles, microtubules of the phragmoplast and the nascent cell wall.

Another phenomenon important to Strasburger was the presence of multinucleate cells in many distantly related plant taxa (Baluška et al. 2012). Nuclei had been known since Brown (1866, first published in 1833) described them, though it was Nägeli who first noted that generally one nucleus was present in each cell (1844–1846), with special exceptions such as pollen and endosperm. Multiple nuclei in ground tissues have more recently been recognized as widely spread phenomena in plants (Prankerd 1915; Beer and Arber 1919) and have been demonstrated for 177 species in 60 families. Though multiple nuclei were first seen by Nägeli (1844–1846) and soon afterwards by Hofmeister (1848a, b, c) it was not for 40 years that their significance for Strasburger was widely recognized, i.e., because they showed that cell division and nuclear division were not necessarily linked.

Most unexpected in Hofmeister's work on pollen development was the use of dilute acid as a histochemical method to coagulate nuclear contents (precipitate chromosomal material, Witty 2015a, b) long before Miescher had isolated and defined the solubility properties of nucleic acids (Miescher 1871; Coleman 1965). Routine visualization of these important organelles and revealing that they

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Figure 6. *Pinus sylvestris*, Scots Pine, engraving for Hermann Karsten (1817–1908), *Abbildungen zur Deutschen Flora H. Karsten's nebst den ausländischen medicinischen Pflanzen und Ergänzungen für das Studium der Morphologie und Systemkunde* (Hermann Karsten's figures for German flora, together with foreign medical plants and supplements for the study of morphology and systematics; Berlin, R. Friedlander and Sohn, 1891). 1. Branch with male and female strobili, *a*; first year, half ripe, *b* and *c*; second year ripe female strobili. 2. Male strobilus magnified. 3 and 4. Male sporophyll from the side and from below (outside). 5. Pollen. 6, 7 and 8. Female sporophyll from the side, back and front. *a*; the scar-free style, *b*; bract, *c*; ovule. 9. Mature carpel with seeds. 10. With a seed. 11. The same without wings. 12. The same, longitudinal section. *a*; Protein in the middle of the seedling.

were a universal phenomenon in plants was a vital first step for theories that explained chromosomes and cell division. Chromosomes must be revealed and counted before concepts of equal distribution in mitosis and halving in meiosis can be formulated (Strasburger 1894). Hofmeister found it important to extend his observations of pollen in flowering plants to the more distantly related Pinaceae (Fig. 6), showing that his ideas were generally applicable to all plants, rather than to a few families, and were at the foundation of botany.

Translation of works like this involved gaining some knowledge of 19th-century German and overcoming conventions that were familiar to Hofmeister and his contemporaries but obscure to us. Most difficult was the practice of using extremely abbreviated references and confusion over "editions" making them very difficult to find, unlike today where particular care is taken over citations. The *Botanische Zeitung* (Botanical newspaper) really was a newspaper and, therefore, has a similar ephemeral quality to the modern internet, i.e., broken URL links. Sometimes I could track down the reference and cite it (e.g., Nägeli 1844–1846), often I could not. German botanical jargon has been recorded for the early (Artschwager and Smiley 1921) and late 20th century (Cole 1994) in brief dictionaries. However, the German language has changed to some degree since 1848. Though I have tried to translate the sense of Hofmeister's work and his conclusions, if I have not succeeded perhaps the reader and I may disagree in this way:

> Books, *and* Dishes *have this Common Fate*; *there was never* any One, *of* Either *of them, that pleas'd* All Palates. *And, in Truth, it is a* Thing *as little to be* Wish'd for, *as* Expected; For, *an Universal Applause* is at *least Two Thirds of a Scandal. So that though I deliver up these Papers to the Press, I invite no Man to the reading of them: And, whosoever Reads, and Repents; it is his Own Fault. To Conclude, as I made this Composition Principally for my Self, so it agrees exceedingly Well with My Constitution; and yet, if any Man has a Mind to take part with me, he has Free Leave, and Welcome. But, let him Carry this Consideration along with him,* that He's a very Unmannerly Guest, that presses upon another Bodies Table, and then Quarrels with his Dinner (adapted from Roger L'Estrange and cited in Campbell 1969).

On the development of pollen

By W. Hofmeister

(Conclusion)

At the end of autumn I examined young anthers of *Pinus* (species *maritima* [*Pinus pinaster*] and *sylvestris*) and *Abies balsamea*, which appear as short and spatulate at the bottom of convex scales. On its underside, near the base, there are two egg-shaped elevations: the protruding anther compartments.

Each anther compartment is filled with a firm cohesive tissue of rather large soft-walled cells, the pollen mother cells. — Each of these cells contains a spherical nucleus with a fairly transparent liquid content and several very small nucleoli, occupying about half of the cell space. The remaining cell space is occupied by gelatinous mucus in which many very small starch granules are embedded. Tincture of iodine colored that mucus only slightly yellow. The coagulated contents, the liquid of nucleus, however, stained deep brown. Two layers of plate-like cells form the entire envelope of the mother cells with most mass to the outside; the layer of horizontally elongated cells, which occurs in so many monocotyledon and dicotyledons, is completely missing in the conifers.

The mother cells remain in the state described during the winter. At the onset of warmer months — in *Abies balsamea* the end of March, for *Pinus sylvestris* the beginning of April and *P. maritima* mid-April, the connections of mother cells dissolve; the membranes thicken, one or two of the nucleoli increase in size. In *Abies balsamea* this growth is far more significant than in *Pinus* where sometimes all trace of nucleoli disappear. — The liquid content of the nucleus coagulates very easily on exposure to water, even faster than in *Tradescantia*. The nuclear membrane is then seen with particular clarity. — Soon afterwards the nucleolus and/or the nuclear membrane dissolve in the same way as *Tradescantia*, *Lilium*, *Iris*, *Passiflora* and the like. Soon two big flattened elliptical nuclei form in the same liquid content of the cell. Their liquid substance refracts light in almost exactly the same way as the liquid contents of the cell, and in the beginning there is no prominent enveloping membrane, and they can be distinguished only with great difficulty. At their first appearance, these nuclei never contain nucleoli; only later, coinciding with the appearance of the surrounding membrane, especially in *Abies balsamea*, nucleoli appear, always in large numbers, up to 20. The nucleoli spilled from the nucleus of a bursting mother cell of *Abies balsamea* stained blue when treated with dilute tincture of iodine; they proved to be similar to starch granules. —

The numerous starch granules of the cell sap accumulate, after formation of the two secondary nuclei, as an annular belt at the equator of the cell. Soon, this belt is divided into two, which are parallel to one another, and this indicates to me, separation of the cell membrane into two halves. These states are found so many times that I have no doubt that they must be gone through by all mother cells. Further development takes place in two different ways, however. Either — this is the much more rare case — a delicate line suddenly

appears at the equator of the cell, between the two particles belts, which under the influence of strong acting reagents immediately disappears. I think it is harmless for the two surfaces of cell membrane resulting from the division of the mother cell to touch. Shortly thereafter, the mother cell has a circular strip running at its equator forming the dividing wall of the two developing special mother cells of the first degree; — or — this is the shelf, — the incipient division of the cell membrane is interrupted by the fact that the membranes of the secondary nuclei are absorbed, and at this site two then four perfectly spherical nuclei soon form. Between any two of these plate-like clusters of granules a delicate line suddenly becomes visible — the first intimation of the two special mother cell dividing walls.

If two special mother cells of the first degree form, then there is the same result every second division, which is that all four cells of a complex of special mother cells form.

The separation of the spherical appendage of pollen cells of *Pinus* and *Abies* takes place while the special mother cells are still inside the wall of the mother cell, which is puffed out towards the exterior. At the beginning both spheres are glassy and transparent with a finely grained surface. The net-like depiction, the yellowish color of the whole mass, occurs later.

In *Juniperus* and in *Thuja occidentalis* the phenomena are entirely the same as above. The observation of the contents is made very much more difficult in the former by the smallness of all its parts and in the latter by numerous starch granules.

Explanation of figures

1. *Passiflora caerulea*. Two mother cells from a 1‴ long bud.
2. The same. A more developed mother cell (both preparations of anther cross sections). — The cell is from one of the outermost strands of mother cells; later on the boundary of this strand is an enveloping slimy layer outlined on its upper side by the developing cells.
3. *P. alata*. Mother cell, at the time when the relationship of mother cells to the lower side has ceased. The cell membrane is exposed as a spherical bubble in the cell space.

3b. The same mother cell after two seconds in water. The cell membrane has expanded and adhered to the cell wall at all points.

4. *Passiflora kermesina*. A part of the cell membrane has emerged from a tear in the ruptured mother cell and has transformed into a large spherical bubble. The nucleus of the mother cell has escaped through the tear and is in the protrubing bubble. The arrow indicates the direction of flow leading from the cell space.
5. The same kind. A mother cell whose nucleus is reabsorbed. It has burst, a part of the cell membrane has emerged from the gap of the wall and by releasing the tension holding the gap open, has been cut off. This process has been repeated two times.

Figs. 6–14. Mother cells of *Passiflora caerulea*.

6. In the mother cell are two water bright bubbles — the first indication of the developing nuclei.
7. Mother cell with three free secondary nuclei; two are large and elliptical, one small and spherical.
8. The cell membrane is a spherical bubble free in the cell space. Two nuclei; one with four, the other with three nucleoli. Between them a delicate, horizontal line; the contact surface of the membranes of the two halves of the cell membrane.
9. Similar appearance of a cell whose cell membrane closely abuts the inner wall of the cell.
10. Mother cell with 9 nuclei. Five of them have emerged from a tear in the wall.
11. Cell with five free nuclei. Between every pair of them plate-like accumulations of grains form.

12. A mother cell, in which the first trace of a projecting strip on the inner wall is to be noted.
12b. The same, half-dehydrated. The cell membrane has withdrawn a little from the inner wall and now appears completely smooth and shows a slight indentation at its equator.
13. Complex of two special mother cells. The cell membranes are exposed in the cell space.
14. A similar complex at a slightly later stage of development treated with dilute sulfuric acid. In the top, two second-degree special mother cells are formed; two nuclei are free in the lower.
15. Complex of 4 special mother cells, only 3 are visible. In the vicinity of one the wall of the mother cell has burst, and the membrane of the special mother cell has emerged as an elongated bubble.
16. Complex of three special mother cells, in each of which has arisen a pollen cell. Two have ruptured their enveloping membranes, and the pollen cells escaped.

Figs. 15–23. *Pinus maritima.*

17. Anther, end of November, from the bottom.
17b. The same, obliquely from above. The right lying locule shines through the layers of cells covering it.
18. The lower part of a longitudinal section of such an anther. The bisected anther compartment and the nearest cells are shown in detail. Only one layer of cells separates the mass of mother cells from prosenchymatous cells of connective tissue.
19. Two mother cells from this anther, more strongly magnified.
20. An isolated mother cell at the end of March with a large nucleus and a small nuclear granule.
20b. The same after a few seconds incubation in water. The liquid content of the nucleus is coagulated.
21. The membrane of the nucleus is reabsorbed and the mother cell contents are like water-clear liquid; the few starch granules are located near the cell wall.
22. Two secondary water-clear nuclei have formed, and the starch granules of the contents form a belt at the equator of the cell.
22b. The same, obliquely from above, to show the ring-shaped arrangement of this belt.
23. The granule belt has become separated into two.
24. Between the two is a delicate line.
25. A projecting circular strip corresponding to the course of that line appears on the inner wall of the cell.
26. Isolated mother cell from *Abies balsamea*, in early March. The nucleolus is rather large.
27. Later state, two secondary nuclei.
27b. One of these nuclei, escapes the bursting mother cell. The nucleoli of the same, stained by exposure to iodine, are blue.
28. Four free secondary nuclei of the second degree.
29. The four special mother cells have formed.

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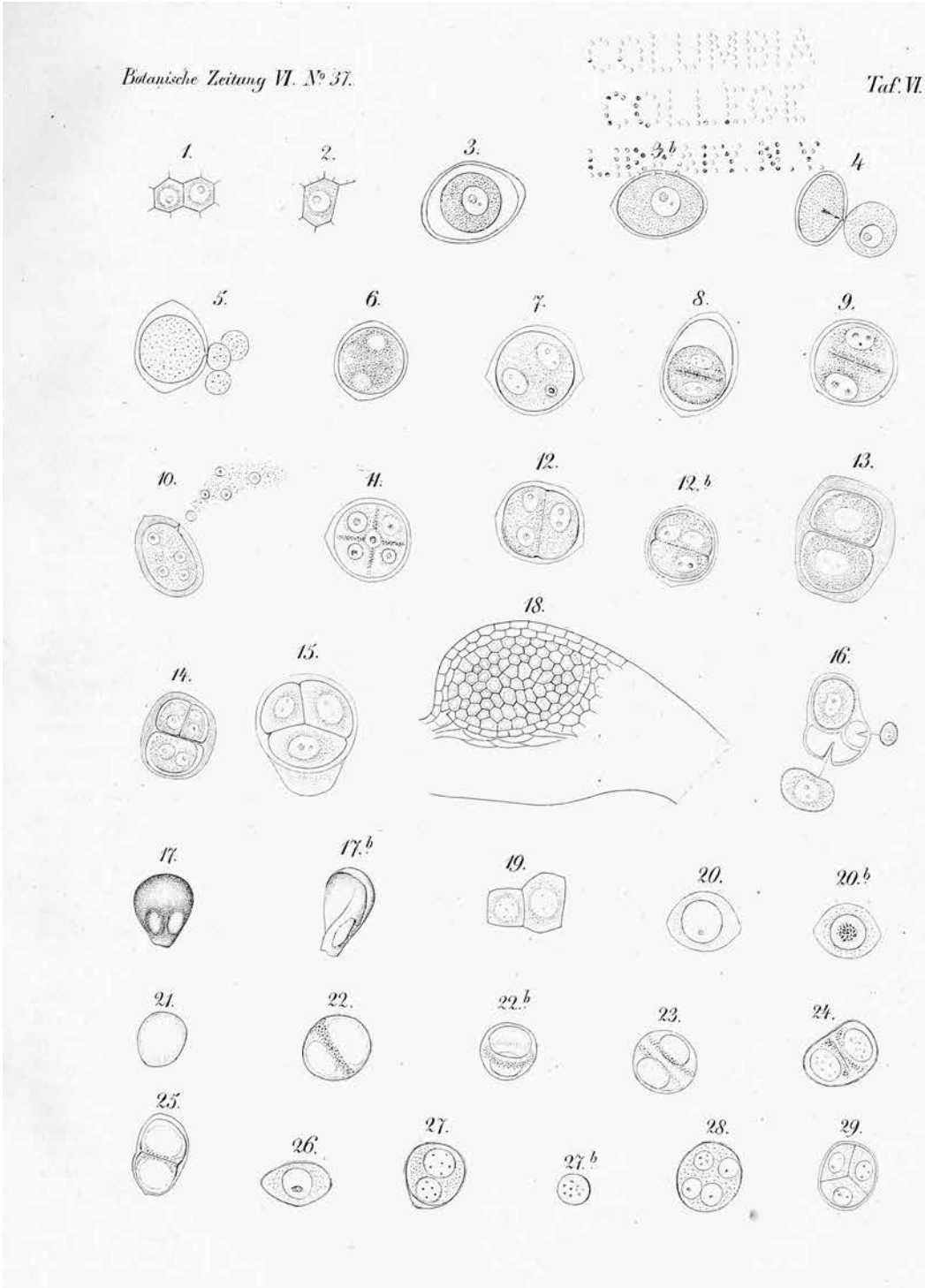
Figure 7. *Right*, Plate 6 of Hofmeister 1848b. Image courtesy Biodiversity Heritage Library (http://www.biodiversitylibrary.org) and the LuEsther T. Mertz Library at the New York Botanical Garden.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