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POLLEN GRAINS

Their structure, identification and significance in science and medicine

BY

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First Edition

McGRAW-HILL BOOK COMPANY, Inc.
NEW YORK AND LONDON
1935

McGRAW-HILL PUBLICATIONS IN THE AGRICULTURAL AND BOTANICAL SCIENCES

EDMUND W. SINNOTT, CONSULTING EDITOR

POLLEN GRAINS

THE MAPLE PRESS COMPANY, YORK, PA.

To

ROBERT A. HARPER

PREFACE

The morphology of pollen grains is as old as most other branches of botanical science; historically, we find that it started at the same time as those which require the use of the microscope, but, through the years which followed—and, indeed, until very recently—it has been neglected to such an extent that it has lagged far behind all other branches of botanical science. At the present time the discovered is but a small part of the discoverable in pollen morphology. The present work, therefore, cannot be a compendium of much knowledge, if, indeed, such were desirable. Though it presents as far as possible what is known about pollen grains, it does so primarily with the object of bringing out the principles involved in their study, of showing where new discoveries may be made, and of furnishing a reliable method of approach.

In the preparation of the material, I have written the sections regarding the pollen grains of the different families and those regarding the evolutionary tendencies as I came to them separately and, for the most part, independently of each other. Consequently they may be read in the same way. Nevertheless, as the different families were finished it was found that they would fit together, quite naturally, in a sequence corresponding, for the most part, to the system of Engler and Prantl. In a few instances, however, in which continuity of the story of the pollen-grain forms demanded a deviation from this sequence, concession was made to the morphology of the pollen grains. Though the story presented here is only fragmentary, its underlying continuity will be better appreciated if approached in the sequence in which it is presented. And this will have the added advantage of emphasizing the huge gaps that remain unfilled and so lend stimulus to their filling which is, perhaps, the most valuable purpose of this book.

In selecting the species of pollen for study, I have kept in mind those of my readers who wish to be able to identify pollen found in the air—and possibly causing hayfever—also those who study pollen fossilized in peats and other sediments. I have included most of the wind-pollinated species known to me as such; also I have included such insect-pollinated species as are known or believed to contribute to the production of hayfever and those which are likely to be found in the fossil records. Nevertheless, the number of species included has had to be greatly extended beyond these limits; otherwise the primary purpose of these studies would be defeated, because morphology is a comparative science. A true understanding of the form of a pollen grain cannot be gained without comparing it with its allied forms; and any deductions drawn concerning it are likely to be misleading without such comparisons. Particularly is this true of the grains of wind-borne pollen, since they are almost invariably reduced in form. Therefore, wherever an anemophilous species is selected for study, as many as possible of its allies are also included. Frequently these are rare and often grow in distant countries, but they are included for the sake of completing the story.

The materials upon which the story of the morphology of pollen grains is based are presented in the specific and, to a certain extent, in the generic descriptions. Like similar descriptions in ordinary botany manuals, these constitute a catalogue of pollen grains, together with the characters by which they may be recognized, and are intended only for reference. In presenting this part of the material I have adopted the language of the taxonomist, characterized by its terse and formal brevity. It is fitting that these descriptions should be couched in the language of the taxonomist, for they rightfully belong to his field; pollen grains are as much a part of the plant as the various organs upon which he has drawn to build his imaginative and surprisingly beautiful classification. But in this he has consistently ignored the pollen grains. In his rejection of them he has thrown away, perhaps, the richest part of his heritage, for in no other part of the plant are to be found packed in so small a space so many readily available phylogenetic characters.

In the discussions of the families are described the form of grain that is basic for the family and from which the others might have been derived and, as far as known, the interrelationships of the various forms to each other and the evolutionary

tendencies manifest in the group. The story as a whole is, therefore, best approached through a consideration of the family discussions, with reference to the generic and specific descriptions only when necessary.

Keys to the genera and species are provided wherever this is deemed advisable. These are not intended for identification, though it is conceivable that in some cases they may be used for such a purpose. They are summaries of the distinguishing characters. The same data could have been presented in tables, but the key is deemed preferable, because it eliminates repetition, and data so presented are more compact and readily available. Though these keys must not be regarded as natural, I have taken advantage of the great flexibility inherent in keys to bring out the characters according to their relative importance and to arrange the different forms according to their natural sequence as far as the limitations of the available data permit their understanding.

The illustrations are all original and entirely the work of the author, except where otherwise indicated. All drawings, except the diagrams, are freehand from studies made with a Zeiss binocular microscope, generally using a magnification of 900 or 1,300, obtained with a 3-mm. apochromatic objective, n.a. 1.4, or with a 2-mm. apochromatic objective, n.a. 1.3, together with paired 15 × oculars.

The greater part of both the written material and the illustrations was prepared especially for this book and appears here for the first time. To the following journals, however, I am indebted for permission to reprint parts of my articles which have already been published: *Bulletin of the Torrey Botanical Club*, *American Journal of Botany*, *The Scientific Monthly*, *The Journal of Allergy*, and *Hygeia;* and to *Practical Microscopy* for permission to reprint some of the figures used in my article on the "Shapes of Pollen Grains," which was published therein.

I am also greatly indebted to many friends for help in various ways and am pleased to take this opportunity of expressing my thanks and appreciation. Particularly is this true of Prof. R. A. Harper, without whose encouragement and kindly criticism this book might never have been written. To Prof. F. A. McClure of Lingnan University, Canton, China, I am indebted for the pollen of *Glyptostrobus;* and to Dr. I. B. Pole-Evans of Pretoria, South

Africa, and to the Royal Botanical Gardens, Kew, for the pollen of *Welwitschia*. The pollen of plants which grow locally was collected by the author, but practically all other material used in the preparation of these pages was obtained from the New York Botanical Garden, and I am indebted to Dr. E. D. Merrill, the director in chief, for many courtesies in the use of the herbarium and library.

R. P. WODEHOUSE.

YONKERS, NEW YORK,
September, 1935.

CONTENTS

LIST OF PLATES

INTRODUCTION

We are accustomed to think of pollen as the fertilizing dust of plants, the yellow powder that forms in the middles of most flowers. In fact, the name pollen just means dust. But, as seen with the microscope's eye, it is a vast assemblage of independent organisms. There are hundreds or thousands of them in the least smear of pollen, but each one is an individual and as much entitled to individual consideration as the plant which produced it.

Pollen grains are small, so small that they cannot be seen with the naked eye; nevertheless, each is a phase in the life cycle of the flowering plant, the point in the cycle where all the potentialities and characters of the plant, be they the beauty of the rose, the majesty and dignity of the oak or the grace and elegance of the vine, are distilled into a tiny cell and cast with millions of others to the winds.

Pollen Grains Are Spores.—They correspond to the small or male spores of the ferns. It is even a little difficult to draw a hard-and-fast line between pollen grains and other kinds of spores. Perhaps the best distinction is this: When a spore, like the fern spore, germinates, it ruptures its wall, and the developing prothallus emerges; when a pollen grain germinates, the young prothallus, which is only rudimentary does not rupture the spore wall but is entirely contained within it. Of course, the word germination, used in this sense with pollen grains, has nothing to do with tube formation, which takes place later. Though the latter is also called germination, the two processes are quite distinct, and the word germination would better be reserved for the first division of the protoplast in the formation of the prothallus.

Pollination.—The problem that confronts pollen grains is that of reaching the pistil of another flower. It is well known that it is toward the achievement of this result that the many different colors and structures of flowers have been developed; it is likewise true that to the same end many of the varied and beautiful forms of pollen grains have been evolved.

Wind Pollination.—Evidently the most primitive method of pollination was the simple and direct method of dispersal by wind. Since pollen grains are ordinarily small enough to float almost indefinitely in the air, provided that it possesses any movement at all, they may simply be cast off on the chance that some will reach their goal. The chances are extremely slight that any one pollen grain will ever reach its destination in another flower by this method, so when wind is the agency employed, the remoteness of the possibility must be compensated by the liberation of an enormous amount of pollen. The most primitive flowering plants are all wind pollinated, *e.g.*, the cycads, the maidenhair tree, and the pines and their allies. The method was eminently successful, for all three of these groups of trees came to dominate the plant societies of past geological ages, though now they have for the most part dwindled to mere fragments of their former grandeur. But the method is extravagant; it is safe to say that for every grain which reaches its goal there are many thousands which are carried astray. Indeed, it is these misdirected wanderers that are the cause of most hayfever.

It is not exactly known how the angiosperms came to abandon wind pollination, but it was probably because insects at some very early period developed the habit of feeding on pollen. This we may see going on today. For example, the corn plant, which is wind pollinated, is freely visited by bees which rob it of its pollen. The gymnosperms, like the maidenhair tree and the pines, appear to have been able to fortify their pollen against this menace, possibly by producing unpalatable pollen or producing it so fast that they get it matured and shed before the insects can breed their numbers up sufficiently to devour it. This appears to be the primary reason why the majority of anemophilous plants have only a brief flowering period.

Insect Pollination.—Other plants appear to have turned this adversity to their advantage; instead of combating the onslaught of insects, they encouraged their visits because the uneaten pollen which they accidentally carried from flower to flower proved to be quite as effective in achieving pollination as vast quantities discharged into the air. Through a long process of evolution, the plants appear to have developed nectar to supply the insects, that they might spare their pollen, and by concealing the nectar in devious ways and placing the pollen at certain vantage points in the flowers much pollen was saved at the cost of only a little nectar. But in order that this method may succeed it is necessary to have the right kinds of insects visit the flowers at the right time. It is obviously to secure this end that the elaborate and beautiful structures of flowers have been developed, always directed towards the most effective pollination with the minimum expenditure of pollen.

Successful as the method of pollination by insects has proved to be with many thousands of plants, undoubtedly success has not been universal. Plants with flowers specialized to pollination by a limited number of kinds of insects must inevitably be restricted in their range to that of the insects which can pollinate them. Their number must, likewise, be restricted by the size of the population of the particular kind of insects required for pollination, and this, in turn, may be limited by the available food supply for the larvae of the insects, or by their natural enemies. Also, sometimes even the flowers most elaborately devised for securing the visits of only beneficial insects must have failed. If we examine almost any patch of squirrel-corn, for example, a large number of the flowers will be found to have been bitten open by some insect which, being unable to secure the nectar in the normal way, has adopted the method of biting a hole in the side of each spur. Also, when bumble bees visit these flowers and thrust their large heads in at the tiny orifice they split the flower wide open, apparently without effecting pollination. So this flower, which is highly specialized for insect pollination, has apparently failed to a large extent, having at least two kinds of insects which defeat its elaborate devices for preserving its nectar for only those insects which can effect pollination.

Return to Wind Pollination.—So we are not surprised to find that during the evolutionary period when the majority of flowering plants were developing many marvelous and beautiful adaptations to insect pollination, to others it became necessary to abandon insect pollination and return to the old extravagant method of wind pollination. To this category belong the families of the grasses and sedges and the majority of, if indeed not all, other angiosperms which are wind pollinated. The flowers of the grasses and sedges possess what are believed to be vestiges of petals and sepals, which seem to show that they are related to the lilies and amaryllis. Having given up insect pollination, they no longer have need of the showy colors of their ancestors and have abandoned them. But they were successful upon their return to their old ways; the grasses cover many square miles of prairies and plains, which, it seems, would be impossible for them to do if they were compelled to rely upon insects for their pollination. The enormous hordes of insects that would be required to pollinate the grasses of the Kansas prairies or the Texas hills or even all the grasses of an ordinary hayfield are almost unthinkable.

Wind-pollinated flowers can generally be distinguished from those that are insect pollinated by their lack of qualities attractive to insects—such as color, perfume, and nectar—, by the relatively much larger quantity of pollen which they shed, and by their protruding anthers designed to scatter the pollen to the wind. Associated with wind pollination there is also generally found a more or less complete separation of sexes, the stamens and pistils being born in different flowers, and the staminate and pistillate flowers on different parts of the plant or even on different plants. For example, the wind-pollinated ragweeds bear staminate flowers in spikes with the pistillate flowers at their bases, and the cattails bear the staminate and pistillate flowers both in the same long spike but separated from each other. A more complete separation is found in the ash and poplar trees, in which each tree bears only staminate or only pistillate flowers.

In their pollination the poplars and willows offer an interesting comparison. They both belong to the same family and are certainly closely related. The willows, however, differ from the poplars in being primarily insect pollinated, possibly in the transition stage, on the way to becoming wind pollinated, while the poplars are entirely wind pollinated. The flowers of both are borne in catkins, the male and female on different trees. Those of the willow, however, are yellowish, sweet scented and provided with nectar, and they prove attractive to insects for they are visited by them in large numbers, while the flowers of poplars are much less conspicuous, without fragrance or nectar, and are of no interest to insects. Though the willows certainly secure pollination by insects, they just as certainly secure it also by wind. They produce an unusually large amount of pollen, for insect-pollinated plants, and if insects fail to carry it away, it is scattered freely in the air. It can easily be detected by appropriate means several miles from the trees, and sometimes

occurs in sufficient abundance to cause hayfever. It is quite possible, therefore, that willow should be regarded as in the transition stage, changing from insect to wind pollination.

It is a curious fact that plants which have abandoned insect pollination and returned to the method of pollination by wind, though they may seem to have failed in their venture in entomophily, they are in no way failures upon their return to the old method. The fact is attested by the extraordinary success of the ragweeds, the grasses, the sagebrushes, many farm and garden weeds like the pigweeds and lamb's-quarters, and most of our forest trees, which are anemophilous. Certainly they are more successful as wind-pollinated plants than are the pines and their relatives and the maidenhair tree, which stuck rigidly all the time to the old-fashioned method, never trying the experiment of entomophily. Though the experiment in itself was a failure with some, it certainly did no harm to those plants which tried and abandoned it, and may even have endowed them with a superior vigor.

Fertilization.—When the pollen grain reaches the pistil, it has already germinated, in the sense that a fern spore germinates. Though the pollen coats are not broken, and from outward appearance the grain is still a single cell, at this stage there are within a number of cells, either actually formed or represented in some way, corresponding in an abbreviated and sketchy fashion to the fern prothallus. When the grain comes to rest on the stigma of another flower the moist secretion which it encounters there stimulates the prothallus to renewed growth, and a slender tube emerges. There may be special pores provided for its emergence, especially if the outer coat of the grain is thick, or the exine may simply rupture. The pollen tube penetrates the tissues of the stigma and style, finally reaching one of the ovules where it discharges two male gametes which were produced by the prothallus of the pollen grain, and fertilization is effected.

WORLDS OF DIFFERENT SIZES

Perhaps the first question that one is going to ask upon approaching the subject of pollen morphology is: Why are the forms of pollen grains so odd and strange—so different from the things that we are accustomed to seeing in everyday life? It is true they are not all alike; unrelated species can easily be told apart, and often the differences between them are very great. Nevertheless, great as these differences are, they are trifling compared with those that exist between pollen grains and the things that we are accustomed to seeing without a microscope. The microscope shows us another world. And truly this is the answer to our question. Pollen grains belong to a world of another size.

The World of Gravity Walkers.—There is a law of nature that places a handicap on bigness; the larger an animal is the greater its difficulty in moving. Mathematicians have a more exact way of stating this law. They say that the surface area, and the cross-sectional area or strength, of a thing are functions of the square of its linear dimensions, while its volume or weight is a function of the cube of the same dimensions. Among the everyday things that we know, this disproportionate increase of volume or weight over surface area or strength that is encountered as an object increases in length and breadth limits the size of animals that, for example, may walk comfortably bearing their weight on their feet to about the size of an elephant. Animals very much larger, like the whales, must live in an aquatic medium that supports their bulk, for it would be totally unmanageable on land. At the other end of the series, a mouse is perhaps the smallest animal that has weight enough to get traction for its feet; and even it freely uses its claws for that purpose. This is the world of our size, bounded at the top by about the size of an elephant and at the bottom by that of a mouse. It is the world in which we perform most of our activities and to which we are best adapted. We need no lenses to see all of it, and we need no mechanical aids to handle things in it, hence our feeling of being at home in it.

In this world of elephants, men, and mice, animals walk on four or two feet, and the bearing of their weight, particularly among those near the upper limit, is perhaps their most serious structural problem. All beings, both plants and animals, which inhabit this size-world are primarily shaped by the asymmetrical or one-directional pull of gravity. The plant directs its stalk upward against the force of gravity and its roots downward with the force of gravity, while its branches may be symmetrically arranged around the vertical axis; and for the same reason animals differentiate an upper and lower side. But the forces of this

size-world tend to impose a further limitation of symmetry on all beings in it which move; for in response to a motion which is always at right angles to the gravitational pull, front and back ends are likewise clearly differentiated. Nature's tendency to make all things as symmetrical as possible is thus thwarted in all directions except laterally, but here it asserts itself. Thus it is that beings which move in this world are characterized by a right and left or bilateral symmetry, which is the only one possible to them.

Among animals which move in this world, flight is seldom undertaken and is hazardous, except toward its lower limit. It may be called the world of gravity walkers. Within it forms of things may be very different, yet, when compared with those of either the world of larger things, which is perhaps the world of planets and solar systems, or with those of the world of next smaller things, to which the insects belong, they seem much alike.

The World of Easy Flight.—Within this world of smaller things gravity and movement impose the same type of bilateral symmetry, as before, upon the beings which inhabit it, but its lessened effect brings about some curious results. The support of bodily weight is not a problem; instead, the effect of gravity is too weak to give sufficient traction for convenient walking on four or two feet in the usual way. Feet must be provided with suction disks or with hooks. In this world of lessened gravitational effect it is nearly as easy to walk across a ceiling or up a vertical wall as it is along the floor. In it flight is the rule and not the exception, and flight is attended with no hazards. So much is this so, that it may be called the world of easy flight. It is a world in which we are not at all adapted and can play no part without special aids. Even for us to observe most of it, a good lens is a help; and to handle objects in it, forceps and other instruments are necessary. Among its inhabitants insects predominate, for their structure, which is far different from that of elephants, men, and mice, is beautifully adapted to movements within its size limits.

Though the effect of gravity is so far reduced that flight is easy, its accomplishment requires muscular effect. Only the smallest inhabitants of this size-world attempt to float without muscular effort, and when they do they must devise some special floating mechanism, such as the tuft of silk of the balloon spider or the crown of pappus of the thistle seed.

World of Floating and Sticking.—In the world of next smaller size, the one to which spores and pollen grains belong, floating is easily accomplished. No wings are used, because none are needed. It is a world of objects so small that when they are free they float, for the effect of gravity is not strong enough to pull them down against the slightest current of air; and when they touch they stick, for the effect of gravity is not strong enough to pull them loose again. But the lack of gravitational effect has an even more important influence, that is, upon the symmetry of the beings of this world; as a consequence of it, their top and bottom sides are not differentiated. Moreover, they do not have an independent forward motion with its consequent differentiation of front and back ends, and they are, therefore, rarely bilaterally symmetrical, and when they are, it is for reasons of another category. With organisms of this size-world, symmetry, which in nature tends ever to be as complete as possible, has much fuller sway and reaches a much fuller expression. Since the sphere is the most perfectly symmetrical figure, it is not surprising to find that beings of this world are basically spherical; it could, indeed, be called the world of spheres. That is, perhaps, the most outstanding character of pollen grains—they tend to be spherical, and their sculpturing is nearly always of a much higher order of symmetry than the bilateral to which we have become accustomed in our world and in the world of easy flight. Is it any wonder that the forms of pollen grains look odd and strange to us?

The range in size of pollen grains does not exactly coincide with the range of the world of floating and sticking. It extends, perhaps, a little beyond at the upper end but falls far short at the lower, for there are spores which are far smaller than pollen grains and still float freely in the air. The largest pollen grains, as, for example, those of the pumpkin, which are about 200 μ in diameter, are so large that they cannot float easily, but they can stick, with the help of a little oily adhesive, so they travel by sticking to insects. From this size pollen grains range downward to about that of the forget-me-not which is $4\frac{1}{2}$ μ in diameter. But it, too, travels by sticking to insects. It is an interesting fact that among pollen grains only those of intermediate sizes

are the best floaters. Invariably both the very large and the very small are exclusively stickers. Only those between 58 and 17 μ, with one notable exception, are good floaters. The reason that those above this range are exclusively stickers is obvious—they are just a little beyond the size range of easy floating but still within the range of moderately easy sticking. But the reason that those below this size range are also almost exclusively stickers is not so easy to see. It may be that their small size, with its attendant disproportionately large surface area, is a hindrance to them in leaving their anthers or in separating from each other. That this is so seems likely from the exceptional case of the pollen of the paper mulberry. It is air borne, yet its grains are only 13 μ in diameter, which is well below the size range of most air-borne pollen. It is, however, forcibly ejected from the anthers in a rather spectacular manner. If a flowering branch of the paper mulberry is kept in water, its pollen will be seen to puff out from the flowers, like puffs of smoke. Also, the many fungi, whose spores are even smaller—yet are floaters par excellence—are provided with an efficient mechanism for throwing the spores clear of the plant and of each other. The reason that only moderately large pollen grains are floaters is probably to be found in the inability of most plants to develop a satisfactory ejecting mechanism.

Resemblances of Pollen Grains to Protozoa.—Many people have marveled at the resemblances between pollen grains and such minute aquatic organisms as the Radiolaria and Heliozoa. The shells of these animals are generally made of some silicious or calcareous material, chemically entirely unrelated to the exine of pollen grains, yet they appear to bear a quite remarkable resemblance to them. Their basic form is spherical, and their sculptured patterns are similar to those of pollen grains in their symmetrical completeness. The question naturally arises: How do these organisms come to resemble pollen grains, since their shells are of an entirely different composition and they live in an aquatic medium? The resemblance that they bear to pollen grains is due solely to the fact that they live in a similar size-world. They are floaters and stickers without independent movement in an aquatic medium, just as pollen grains are in an aerial medium. And, freed from the asymmetrical influence of a one-directional gravity and of one-directional movement, they have become as nearly perfectly symmetrical as pollen grains, assuming the spherical form and somewhat similar sculptured patterns. Looked at more closely, however, they are seen to have no more resemblance than this; their sculptured patterns are composed of different elements of symmetry, belonging to different mathematical series. For example, the underlying series among the patterns of pollen grains is that of the tetrahedron, cube, and pentagonal dodecahedron, always with three equal angles coming together at a point; while the patterns of the most similar protozoa, *e.g.*, *Circoporus* and *Circogonia*, are built on the plan of the octahedron and icosahedron, which have four angles coming together at a point, a condition almost never encountered among pollen grains. Such differences as these are basic and, measured in terms of the world of floating and sticking objects, are far greater than, let us say, the difference between a man and a crocodile, measured in terms of our world of gravity walkers.

The actual similarity that the inhabitants of the world of floating and sticking usually bear to each other is due to the fact that within the size limits of such tiny objects as spores, pollen grains and protozoa, much less diversity of form is possible than within the worlds of larger objects. It is true that at its upper end and, in fact, through the greater part of its range, which is occupied by pollen grains, considerable diversification of form is possible. The large pumpkin pollen grain, those of the Malvaceae, with a diameter of about 153 μ, and of the four-o'clock, with a diameter of about 180 μ, are elaborate and beautiful objects. Nowhere among the grains of smaller orders of magnitude do we find anything approaching the multiplicity of their detail and beauty of pattern. As we pass downward in the series of grains arranged according to their size, they become less and less ornate. The smaller pollen grains are practically without decorations, excepting germinal apertures of the simplest kind or germinal furrows of an equally simple kind; and as we pass over into the domain of spores which are still smaller even these are missing. Indeed, the smaller spores in their simplicity and plainness of form are quite like bacteria which are the inhabitants of the next smaller size world.

In the world of bacteria and organisms of similar size the forms are all so simple that it is difficult to distinguish most of them by

their morphological characters. For that reason the bacteriologist must rely mainly upon physiological characters for purposes of classification and identification.

We shall not dwell longer upon this size-world, or upon those which lie still lower in the scale, than to point out that as the size of the particle diminishes and, as a consequence, the proportion of the surface area increases, new and unfamiliar properties are encountered, and the old familiar ones lost. For example, the world next in size below that of bacteria is perhaps the world of colloid chemistry. In this world particles are too small for us to see even with a microscope; but if we could see them, they would probably be found to be perfect spheres and all look much alike. The properties which distinguish colloids are mainly those which have to do with surface areas, for truly this is a world of enormous surface areas.

A consideration of objects and organisms of these different size classes furnishes a suitable background against which to view pollen grains. Their most striking and fundamental characters are those of the size-class to which they belong and do not lend themselves to ready comparison with objects of other size-classes. Underlying this is the old familiar law which says that the surface area of an object is a function of the square of its linear dimensions while its volume is a function of the cube of the same dimensions.

PART I

GENERAL

CHAPTER I

HISTORICAL REVIEW

The history of pollen morphology is necessarily associated with the development of the microscope. On account of the small size of pollen grains not even the first beginnings of their study could be made until the microscope had reached a fairly high stage in its development, and each notable improvement in the construction of the microscope has always been reflected in a corresponding advance in pollen morphology. Though the microscope as we now know it is distinctly a modern instrument, its origin is lost in antiquity. There is even some evidence that the ancient Greeks had simple lenses and understood the principles of magnifications, but not until the middle of the seventeenth century, when Hooke gave the world his compound microscope, was an instrument constructed of sufficient power to reveal anything of the shapes of pollen grains.

Hooke's microscope is described and illustrated in the preface to his "Micrographia," which is dated London, 1665. It consists of a tapering brass tube with a single objective at the small end, a large eyepiece at the opposite end, and between these two a third lens could be inserted; but, he states, he did not use this lens, because the fewer the refractions used the clearer the image. The tube was also provided with an opening whereby it could be filled with water, which Hooke states caused the image to appear sharper but was too troublesome to use.

Though such an instrument would appear crude and useless to us, it is hard to estimate its importance in the seventeenth century. From it emerged a whole host of discoveries. The work that Hooke himself did with his microscope, now preserved in his "Micrographia," was in many ways remarkable, but it was too discursive and entirely lacking in unity of purpose, except possibly to demonstrate the beauty and achievements of his microscopes, of which he seems to have been justly proud. But of much farther reaching effect was the work of his contemporary microscopists Malpighi, Grew, and Swammerdam, who with van Leeuwenhoek, using a simple microscope, were really the founders of microscopic anatomy.

Of these, only Grew and Malpighi have had anything to say about pollen. Almost simultaneously they observed and described pollen grains, pointing out that, while those of the same species were alike, those of different species were different. It is

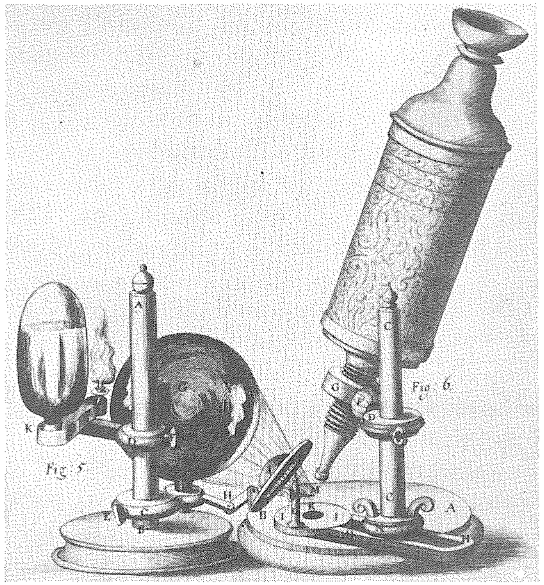

FIG. 1.—Hooke's microscope.

not a matter of any importance to us which of them was the first to describe pollen grains, though most historians seem to think that it is incumbent upon them to settle the question of the priority of these two men, and much bitterness has resulted from the fruitless discussions centering around this question. Though Malpighi lived in Italy, and Grew in England, each was quite familiar with the other's work and borrowed freely from the other but always with acknowledgment as complete as was customary in those days. The question of priority seems not to have interested them at all then—and no more need it interest us now. Malpighi and Grew are recognized as the cofounders of microscopic plant anatomy and so it is that they are cofounders of pollen-grain

morphology. But, the contribution of Grew to the latter greatly outranks that of Malpighi, though possibly the reverse is the case with their contributions to plant anatomy in general. The difference between the work of the two men is perhaps more a matter of character than of merit. And, though they were cofounders of pollen morphology, it will perhaps be better to discuss their lives and work separately.

Nehemiah Grew.—Nehemiah Grew was born in 1641 at Atherstone, Warwickshire, the only son of Obediah Grew, a schoolmaster who afterward became the nonconformist vicar of St. Michael's Coventry, where Nehemiah spent his youth. Grew's life extends through some of the most troublous times that England has ever experienced. Born the year before Charles I proclaimed war upon the Parliamentary forces, he witnessed the fall and execution of Charles I, the establishment of Cromwell as Protector, and the reigns of Charles II, James II, William and Mary, and the greater part of that of Queen Anne. Yet all the turmoil going on about him seems to have left his life singularly unruffled. He attended Cambridge University, where his acquaintance with John Ray first stimulated his interest in botany. On leaving Cambridge, Grew went to Leyden, where he received the degree of M. D. in 1664, after which he returned to Coventry to practice medicine.

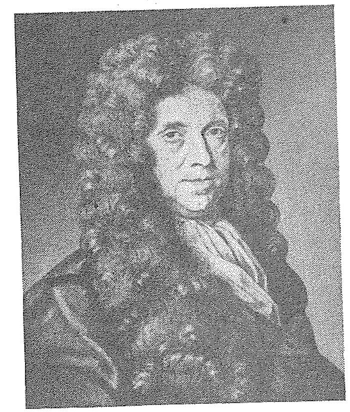

FIG. 2.—Nehemiah Grew (1701) after the portrait by R. White.

He turned to the study of vegetable anatomy and physiology in the hope of gaining a clearer understanding of the anatomy and physiological processes taking place in the human body, because, he says: "Upon reading some of the many curious Inventions of Learned men, in the *Bodies* of *Animals*. For considering that both of them came first out of the same *Hand* and were therefore the contrivances of the same wisdom I thense fully assured my self that it could not be a vain Design; to seek it in both." And indeed all of Grew's work is colored by this conception of the analogy between animals and plants in their structure and physiological processes.

All Grew's botanical work is bound in a single volume—"The Anatomy of Plants," published in London in 1682, though some of it had been published 11 years earlier. The first of his essays, "The Anatomy of Vegetables Begun," was published by the Royal Society of London in 1671, the same year that Malpighi's "Idea" was submitted to the Royal Society in manuscript form. Since the present discussion is concerned mainly with the history of pollen morphology, it is necessary to refrain from going deeply into this great work, which in many ways seems amazingly modern. Agnes Arber, in her delightful essay in "The makers of British Botany" (1912), truly says:

> It is no less than two hundred and forty years since Grew sent his first treatise to the Royal Society, so it is scarcely wonderful that a number of his results have been rejected in the course of time. It is far more remarkable that far more of his conclusions—and those the more essential ones—have been merely confirmed and extended by later work.

In his description of the flower, after describing in a manner which would do credit to a modern textbook of botany the various ways in which the anther may be articulated with its filament and the ways in which dehiscence may take place, he says: "At these clefts it is that they disburse their powders; which as they start out, and stand betwixt the two lips of each cleft have some resemblance to the common sculpture of a Pomegranate with its seeds looking out at the cleft of its Rind." In speaking of the anthers elsewhere, he says: "These parts are hollow; each being the Theca or Case of a great many extreme small Particles either globular or otherwise convex. . . . They are all crowded together and fastened in close ranks without any pedicils, to the sides of the Theca." His descriptions are always concise and vividly pictorial in effect. Knowing full well that his reader was unlikely to be possessed of a magnifying glass and totally ignorant of its possibilities, he compares what he saw through his glass with familiar objects seen with the unaided eye:

> The Particles of these powders, though like those of Meal or Dust, they appear not easily to have any regular shape; yet upon strict observation, especially with the assistance of an indifferent Glass, it doth appear, that they are a Congeries, usually of so many perfect Globes or Globulets; sometimes of other Figure, but always regular. That which obscures their Figure is their being so small: In Dogs-Mercury, Borage, and very many other Plants, they are extremely so. In Mallows, and some others, more fairly visible.
>
> The Colour of these small Particles contained in the Theca is also different. But as that is usually white or yellow, so are these: sometimes Blewish; but never Red. And sometimes not of the same Colour with that of the Theca. Which further shows how scrupulous Nature is in differentiating the Tincture of the several Parts.
>
> They are also of different Bigness and Figure. Those in Snap-dragon, are of the smallest size I have seen; being no bigger through a good Microscope, than the least Cheese-Mite to the naked Eye. In Plantain, also through a Glass, like a Scurvy-grass-Seed. In Bears-foot, like a Mustard-seed. In Carnation like a Turnip-seed. In Bind-weed like a Pepper-Corn. In all these of a Globular Figure.
>
> In Devils-bit, they are also round, but depressed, like the seed of Goose-grass, or a Holland cheese. In the Bean and all sorts of Puls, and Trefoyls, also in Blew-bottle, &c. they are Cylindrick. In Orange Lilly, Oval, one 5th of an Inch long, like an Ants-Egg. In Deadly-Nightshade, also Oval, but smaller at both Ends. And those of Pancy, Cubick. In all these and the former, they are Smooth.
>
> But in Mallow, Holyoak, and all that Kind, they are beset round about with little Thorns; whereby each looks like the Seed-Ball of Roman Nettle, or like the Fruit of Thorn-Apple, or the Fish called Piscis arbis minor, or the Murices, used anciently in Wars. They are also very great, showing, through a Glass, of the bigness of a large White Pease; being 200 or 300 times biger than those of Snapdragon; of which there are about a Thousand in each Theca, that is in the space of about 1000th Cubical Part of an Inch.

The grains described above are all illustrated in a plate that follows their description; but the figures are disappointing, falling far short of his graphical descriptions, and in no way bear comparison with his anatomical drawings.

Whether or not Grew understood the fertilizing function of pollen is not quite certain. He is sometimes credited with priority in the sexual theory of plants, since he stated somewhat vaguely that he believed that fertilization was one of the several functions of pollen. However, the credit for its discovery belongs more properly to Camerarius, who some years later (1694) established with abundant evidence the existence of sexuality in plants. The question of the usefulness of pollen clearly caused Grew considerable concern. He feels that the beauty of the petals and sepals of the flowers is their justification. And this, he thinks, may also be true of the stamens. But, if so, why do their anthers split open? For this really destroys their beauty. But he noticed that flowers are nearly always occupied by insect "guests." So, attributing to flowers the same generosity of soul that he himself is known to have possessed, he concludes that the pollen grains in the anthers are to provide food for "these vast numbers of little animals," each flower "thus becomming their Lodging and their Dining-Room both in one." Yet he is not quite sure whether they "only suck from hence some juices . . . or also carry some of the Parts, as of the Globulets, wholly away." It is truly quite remarkable how near he came to discovering insect pollination, but this remained for Kölreuter about a hundred years later. That Grew was far from satisfied with his explanation of the usefulness of pollen to the plant is quite certain, for he closes his discussion of the matter with these words: "Or lastly what may be the Primary and private use of the Attire [stamens] (for even this above-said, though great, yet is but secondary) I now determine not." Elsewhere he says: "It would appear that the attire serves to remove some superfluous parts of the sap, as a preparatory process to the production of seed." This idea seems to have been borrowed from Malpighi and is hard to reconcile with his statement, made somewhat earlier in the "Anatomy of Plants," in which he says:

> In conversation with our learned Savilian Professor, Sir Thomas Millington, he told me he was of the opinion that the attire served as the male organ in the production of seed. I replied at once, that I was of the same opinion, and gave him some reasons for it, answering at the same time some objections that might be brought against it.

Though Grew was halting and uncertain in his statements regarding the usefulness of the anthers and pollen, in describing pollen grains he paints the picture with a few bold strokes; and he clearly states that, though pollen grains tend to be spherical or globular in form, they are of different size and form in different species but those of the same species are all alike. In this he may be said to have laid the foundation of pollen morphology.

Malpighi.—Cofounder with Grew of the science of plant morphology, Marcello Malpighi, was born at Crevalcore near

Bologna, Mar. 10, 1628. His parents were farmers, enjoying an independence in financial matters. Studying at the university he became doctor of medicine in 1653 and taught for two years, until he was appointed to the chair of theoretical medicine at Pisa. But, never of robust health, he was forced to leave Pisa on account of the climate after three years, whereupon he went to Messina in 1662 as professor of medicine, returning to Bologna in 1666. In 1667 he was made a fellow of the Royal Society of London, publishing his treatise on the silkworm, which was the first of a long series of papers on zoology, medicine, and botany, eventually collected and published by the society as the well-known volume "Opera omnia" (1687). In 1691, at the age of sixty-three, he became private physician to Innocent XII, but before he had long held this position he died of apoplexy (1694).

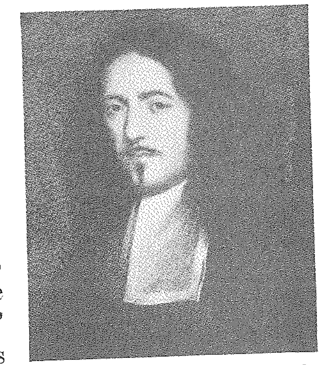

FIG. 3.—Marcello Malpighi after the portrait by Tabor.

"Malpighi was primarily physician, as we must perforce acknowledge, yet nothing, we find, is beyond the curiosity of Malpighi the botanist." He says that he began his studies with human anatomy but being unable to understand what he found there turned to the study of higher animals; he gained from them nothing, so, for the sake of further comparisons, turned to the world of insects and finally to plants in his search for simpler structures, for, as he says, "I thought that if I mastered these I might retrace my steps upwards to the higher forms, and clear the path of my earliest studies."

Though Malpighi was on an equal footing with Grew, or possibly even superior, as the founder of microscopic plant anatomy, his contributions to pollen morphology fell far short of those of Grew. Nevertheless, he revealed the basic principles in much the same way that Grew did. He says that "the chambers of the stamens are packed with a mass of globulets almost atomic." His expression *globulorum congerie, quasi atomorum* is surprisingly like that used by Grew in describing the pollen in the anthers. Continuing, he says, "These are of various color and shape, generally yellow as in the lily and rose, but generally white and transparent in the mallow and plantain. Likewise of various form. That of the lily is distinctly oval but pointed at its ends, and throughout its length, as in a wheat seed, extends a furrow." This mention of the single furrow of the lily pollen is apparently the first observation of the principal characteristic of the pollen of most monocotyledons. The very few figures of pollen that he gives are, like those of Grew, disappointing, but he shows quite correctly the single furrow of the lily grain.

In all his observations Malpighi was obsessed with the idea that every part of the plant had its counterpart, both in form and function, in the animal body, and this led him to the most surprising conclusions, considering his remarkably accurate observations. Regarding the pollen, he says:

> The plant egg or ovum is concealed in the ovary . . . surrounded by stamens and a floral envelope. Aeration of the egg may be facilitated by the hollowness of the style, and the entrance of noxious insects prevented by the secretion of a sticky fluid . . . in this fluid are secreted also the impurities of the sap. The pollen dust is likewise a mere secretion . . . prior to the maturation of the ovum . . . and may be compared perhaps to the menstrual discharge of women.

At the time when Malpighi wrote this, Camerarius had already correctly interpreted the organs of the flower, including the pollen, but Malpighi could not have known it, for Camerarius' work was not published until after Malpighi's death.

An interesting comparison is drawn between the works of Malpighi and Grew by Sachs in his history of botany: "If Malpighi's work reads like a masterly sketch in which the author is bent only on giving the outlines of the architecture of plants, the much more comprehensive work of Grew has the appearance of a text-book of the subject. . . . The tasteful elegance of Malpighi is here replaced by a copiousness of minute detail."

With the passing of these two great pioneers the study of botany stagnated for about 150 years. Possibly it was because these men had reached so far ahead of their time that no one was able to follow them; or it may have been that the capabilities of the microscope, as it existed then, had been exhausted, and the stimulus of the invention of the compound instrument had worn out. Whatever the cause, the science of pollen morphology received no contributions of note until well into the nineteenth

century, when with a sudden re-awakening appeared, almost simultaneously, the works of Mirbel, von Mohl, Purkinje, Fritzsche, and others. Before we bridge this gap, which takes us into work of a decidedly modern aspect, it will be well to pause a little to consider the problem of the sexuality of plants, because of its necessarily close association with pollen morphology.

SEXUALITY IN PLANTS

The Assyrians.—The earliest known recognition of sex in plants is attributable to the ancient Assyrians, who, it is fairly certain, practiced hand pollination of the date palm. Among the many carvings and examples of glazed brickwork that have been recovered from their ancient civilization are a number which have been interpreted to represent this practice. But the theme is always mythological, and its representation so highly stylized as to leave its basic origin uncertain. Thus, in the palace of Ashur-nasi-apal, who was the worshiped deified ruler of the Assyrian empire from 885 to 860 B.C., have been found carved slabs of stone representing this theme. These are described by A. T. Olmstead, who, in his history of Assyria (1923), writes as follows of the carvings which decorated the altars at the gate of the palace:

> The middle one represents eagle headed beings with huge beaks, lolling red tongues, stiff high crests, their dress and posture like that of their more human companions, save that their weapon was a knife stuck into their girdle; their right hand held aloft the spathe which was to fertilize the palm, in the left was a small basket.

Some of the best of these sculptures are preserved in the Metropolitan Museum of Art. These show the same theme repeated again and again, with slight variation, in the different parts of the temple. Always the beings are gigantic, both actually and as compared with the palms which they are believed to be pollinating; always they have large, widespread wings; and always the posture is the same, with the upraised right hand bearing the spathe, which is sometimes smooth and sometimes imbricated, and the left hand hanging at the side bearing a basket. The head is sometimes that of an eagle with partly open beak and protruding tongue and sometimes that of a man with plaited beard, and into the girdle are always thrust two or three knives with the handles showing, that of the third knife, when present,

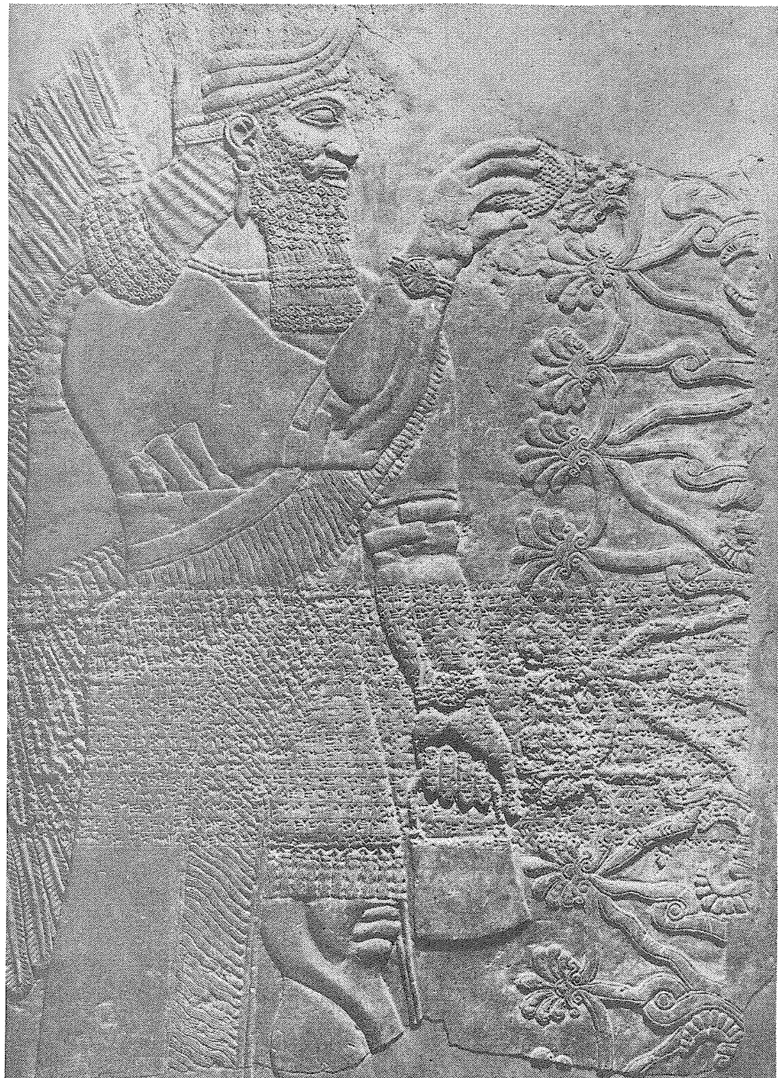

Fig. 4.—Assyrian winged being pollinating the date palm. (*Courtesy of Metropolitan Museum of Art, New York City.*)

bearing the head of an animal. "In the room itself," Olmstead tells us, "were sculptured figures of Ea, the fish god. He, too, raised the fertilizing palm spathe. And over the figures of the king and his chancellor, themselves 8 ft. high, towered to twice the height a gigantic, winged genius with the fertilizing palm spathe." Again, from the palace of Sargon, 717 B.C., has been recovered a beautiful relief in glazed brick of a winged being somewhat similar though differently attired bearing in his hand the same object which is believed to be a spathe and with which he appears to be pollinating a date palm.

Some doubt has been cast upon the interpretation of these figures as representing pollination. L. Legrain states that they "do not represent the pollination of the date palm. The stylized trees, and the mythologic guardians are watching the tree of 'fortune,' happiness, and good luck and keeping the fruits for the king."

Whatever the correct interpretation of the Assyrian winged beings may be, it is recorded that Herodotus,* in the middle of the fifth century B.C., brought back from his travels in the East the information that the date palm is of two sexes and that in Assyria the female tree is fertilized by dusting it with branches of the male. But this was not followed up, for we find Aristotle, the founder of natural history in the middle of the fourth century B.C., categorically denying the existence of sex in plants because they are nonmotile, and he believed that, as in animals, the sexes were separate only in species which were free to move about. His pupil Theophrastus was somewhat better informed, for he at least suspected the truth. He says that terebinths are some male and some female and that the former are barren and are therefore called male. However, he was more of a philosopher than a naturalist and dismisses the subject thus:

> What men say, that the fruit of the female date palm does not perfect itself unless the blossom of the male with its dust is shaken over it, is indeed wonderful, but resembles the caprification of the fig, and it might almost be concluded that the female plant is not by itself for perfecting the foetus; but this cannot be the case in one genus or two, but either in all or in many.

* In this discussion of the early development of the sexual theory I have followed Miall and Sachs.

Pliny.—Little is known of the development of the sexual theory of plants from this time until that of Pliny, about 350 years later, but in the meantime it must have become well established, for Pliny, himself not a naturalist but rather a "compiler of anecdotes," calls the pollen dust the material of fertilization and says that naturalists tell us that all trees and even herbs have the two sexes. After this, for nearly 16 centuries, the question excited little interest among naturalists. The idea was accepted and made use of by Konrad von Gesner and de l'Ecluse, early herbalists of the sixteenth century. Prosper Alpino in 1592 had observed the pollination of dates in Egypt, but Jung and Cesalpino regarded the sexuality of plants as an absurdity, and, as we have seen, Malpighi, who gave the first careful study of the seed and development of the embryo, rejected entirely the idea of sex in plants. The next advance was the simple statement of Sir Thomas Millington, reported by Grew, which we have already seen above. But this was thrown out only as a suggestion, and, while Grew stated that he believed Millington's conjecture that the stamens are the male organs, he did not follow up the suggestion and the other functions which he ascribed to the stamens showed how little the idea of their sexual significance had aroused his interest. Ray, in his "Historia plantarum" (1693), reinforces the conjecture of Millington and Grew by citing once more the example of the date palm. "In the desert," says Ray, "when artificial pollination cannot take place, the wind may possibly answer the same purpose." It remained, however, for Camerarius to put the question to the test.

Camerarius.—Rudolph Jacob Camerarius was born in Tübingen in 1665 and died there in 1721. In 1688 he became director of the botanical garden in Tübingen and in 1695 first professor of the university, succeeding his father, Elias Rudolph Camerarius. Camerarius' work is known to us principally through his famous letter "De sexu plantarum" addressed to Valentine, professor of Giessen, dated 1694, some years after his observations had been made. He observed that a female mulberry tree, growing with no male tree near, bore fruits which contained only abortive seeds. This aroused his interest so much that he took female plants of *Mercurialis annua* and kept them in pots apart from the others; he found that, though the plants throve and produced fruits, not one produced a fertile seed. His

communication on this subject was the beginning of the large number of experiments which were described in detail in his letter to Valentine. He carefully describes the flower, the anthers, the pollen, and the behavior of the ovules and then proceeds to the experimental part. He removed the male flowers of *Ricinus* and found that he got no seeds. From this he concluded that no ovules of plants could ever develop into seeds without first being prepared by the pollen which is borne in the stamens. "It therefore follows," said he, "that the stamens are the male sexual organs in which that powder which is the most subtle part of the plant, is secreted and collected, to afterwards be supplied from thence." Finding that most flowers are hermaphroditic, he concluded that they were always self-fertilizing, but he thought it strange by comparison with fertilization in snails which are hermaphroditic animals. The complete explanation, however, remained for Kölreuter and Sprengel nearly three quarters of a century later. Among Camerarius' many experiments were several failures, which, in his scientific way, he took pains to report. He was much disturbed to find that three plants of hemp taken from the field and planted in the garden produced fertile seeds, and upon repeating the experiment by growing the plants indoors in pots he obtained the same results. In this case it appears that he left to some one else the cutting out of the male plants.

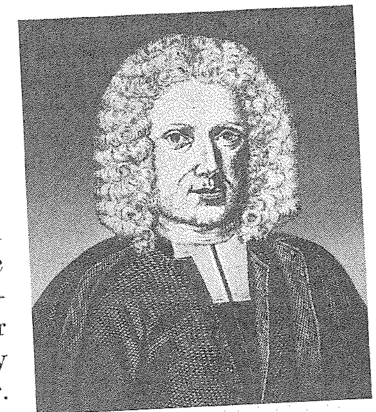

Fig. 5.—Rudolph Jacob Camerarius (1665–1721).

James Logan.—The importance of Camerarius' discoveries soon became appreciated by other investigators. Some denied them; others declared that they knew them all the time; but others were sufficiently aroused to put them to the test. Among the latter was James Logan, governor of Pennsylvania. He set some plants of corn at opposite ends of a plot of ground, about 80 ft. apart. In one group he left the plants intact, but from the others he removed the male panicles. Later in the summer he observed that the ears of the hills in which the plants had been decapitated were all sterile except "one large ear which grew out somewhat further from the stalk than usual, and on that side too, which faced another hillock in a quarter from whence our strongest winds most commonly blew." An ear among the unmutilated group, which had been wrapped in muslin, remained without a fertile seed. It thus appears that Logan may be credited with the first appreciation of wind pollination. The exact date of this work is not known, but it appears to have been published at the Hague in 1739.

Miller.—The credit for the recognition of insects as an agency in pollination appears to belong to Philip Miller. In 1751 he planted 12 tulips about 20 ft. apart and attempted to prevent fertilization by removing all their stamens as soon as the flowers began to open. Some days later, however, he saw some bees load themselves with pollen at an ordinary tulip bed, fly over to the flowers upon which he had operated, and when they had gone he observed that they had left on the stigmas sufficient pollen for fertilization and these flowers later produced seeds.

Gleditsch.—The argument for the necessity of pollination was clinched by a series of masterly experiments by J. G. Gleditsch in 1749. Gleditsch had already observed that the spores of fungi were disseminated everywhere by the air. He had observed that a female date palm (*Chamaerops humilis*) which had been brought to Berlin from Africa had never been known to produce seed, though it was about eighty years old, and he himself had observed it for 15 years. There was no male tree in Berlin, so he procured part of a male inflorescence from a tree growing in Leipzig which he dusted over the female flowers of the Berlin tree and tied on to the inflorescence. The result was that fruit ripened the following winter and germinated in the spring. Though this was only doing once more what Herodotus had reported the Assyrians as doing in the fifth century B.C., it was the only experimental proof since that of Logan of the sexuality of plants and cleared the ground for Kölreuter and Sprengel.

Kölreuter.—It was about 12 years later that the memorable work of Joseph Gottlieb Kölreuter was done. His report, "Verläufiger Nachricht vom einigen das Geschlecht des Pflanzen betreffenden Versuchen und Beobachtungen," was published in

four parts, between the years 1761 and 1766. The work has been summarized for us by Sachs in his history of botany, from which the following account is drawn. Kölreuter recognized the importance of insects in flower pollination. He had observed that many flowers had something within them that was attractive to insects. From this he concluded that flowers which were incapable of pollinating themselves were pollinated by insects. He collected nectar from many flowers and found that upon evaporation it gave a sweet substance like honey; from this discovery he concluded that bees made honey from nectar. In order to discover how effective insects were in the fertilization of flowers, he pollinated 310 with a brush, leaving the same number to be pollinated by insects. The number of seeds formed in the latter case was only a little less than in the former.

His contributions to the morphology of pollen grains were notable, despite the fact that his microscopes were very imperfect. He discovered that the outer covering of pollen grains consisted of two distinct coats and noticed in some species the spines and sculpturing on the outer coat and its elasticity. He observed the orifices and their lids in the exine of pollen grains of *Passiflora*. He noticed that when the grains were made wet the inner coat would protrude through the apertures of these grains, standing out as rounded papillae, which in some cases eventually ruptured and allowed the contents to escape. He correctly interpreted the formation of papillae through the protrusion of the inner coat through the pores as permitting some expansion of the grain without the rupture of the wall. Yet Sachs, in summary, regards this as a mistake on Kölreuter's part, pointing out that these papillae are really the beginnings of pollen tubes of which Kölreuter was unaware. It is true that Kölreuter did not understand the function of pollen tubes in fertilization. It was, perhaps, on this account that he was able to recognize the much more subtle function of the papillae in volume-change accommodation, which is unquestionably a necessary, though perhaps secondary, function, in all those grains which have heavy walls and have no furrows which may accommodate changes in volume.

Gleichen and others thought that the grains contained spermatozoa and must burst to discharge them in order to effect fertilization. But Kölreuter did not regard the bursting of the grains as a natural process, since they obviously possessed devices for its prevention. Instead, he started with the hypothesis that the oil on the surface combined with the moisture of the stigma forming a new substance, in the same way that an acid and an alkali unite to form a salt, and that this substance, if fertilization is to ensue, must be absorbed by the stigma and conveyed through the style to the ovules. But he later abandoned this idea, for experience taught him that if he exchanged the moisture of a stigma for that of an allied species, then dusted the stigma with its own pollen, he got no hybrid.

Kölreuter's most important contribution to the development of the sexual theory was his artificial production of hybrids. He produced hybrids of *Nicotiana, Dianthus, Mathiola, Hyoscyamus*, and others. He showed by experiment that if a stigma received the pollen of its own species and that of another at the same time, only the former is effective, and he drew the logical conclusion that that is why hybrids are rare in nature, though they can easily be produced artificially.

Sprengel and Flower Pollination.—The next important contributions to the sexual theory are the brilliant researches of Konrad Sprengel (1812). He showed that cross pollination was the rule and not the exception and stated that "Since very many flowers are dioecious, and probably at least as many hermaphroditic flowers are dichogamous, Nature appears not to have intended that any flower should be fertilized by its own pollen." Dichogamy, the maturing of the stamens and pistils at different times, had previously been noted by Kölreuter without appreciation of its significance. The importance of insects in pollination was brought out forcibly by Sprengel. He says:

> In the summer of 1787 I was attentively examining the flowers of *Geranium sylvaticum*, and observed that the lower part of the petals was provided with slender rough hairs on the inside and on both edges. Convinced that the wise framer of nature produced not a single hair without a definite purpose, I considered what end these hairs might be intended to serve. And it soon occurred to me, that on the supposition that the five drops of juice which are secreted by the same number of glands are intended for the food of certain insects, it is not unlikely that there is some provision for protecting this juice from being spoiled by rain, and that the hairs might have been placed where they are for this purpose. Since the flower is upright, and tolerably large, drops of rain must fall into it when it rains. But no drops can reach one of the drops of juice and mix with it, because it is stopped by the hairs, which are over the juice drops, just as a drop of sweat falling down a man's brow is stopped by the eye-brow and eye-lash, and hindered from running into the eye. An insect is not hindered by these hairs from getting at the juice. I examined other flowers and found that several of them had something in their structure, which seemed exactly to serve this end. The longer I continued this investigation, the more I saw that flowers which contained this kind of juice are so contrived that insects can reach it, but that the rain can not spoil it; but I gathered from this that it is for the sake of the insects that these flowers secrete the juice, and that it is secured against the rain that they may be able to enjoy it unspoilt.

Sprengel noticed that the markings of petals and their colors could serve to guide the insects to where the nectar was. He observed dichogamy in *Epilobium angustifolium* and found that the older flowers were fertilized by pollen brought to them from the younger. He found the same thing in *Nigella* but the opposite in a species of *Euphorbia*. He showed that in protandrous flowers—those in which the stamens mature first—the exact place which was occupied by the stamens is subsequently occupied by the stigma so as to be in position to remove the pollen from the body of an insect which has just left a younger flower. From such observations he concluded that the whole structure of flowers was an adaptation to secure pollination by one or several species of insects.

Sprengel clearly distinguished between anemophily and entomophily. He showed that all flowers "which are without a proper corolla and have no calyx in its place, are destitute of nectar and are not fertilized by insects, but by some mechanical means as by the wind." He also observed that such flowers produced light pollen and in large amounts, while with insect-pollinated flowers the reverse is the case. He showed that all the devices of flowers, whether for insect pollination or for wind pollination, pointed indubitably to the fact that nature avoided, as far as possible, self-pollination. It remained only for Knight, Herbert, and Gärtner to show that crossing of flowers produced more numerous and more vigorous progeny and that repeated self-fertilization led to a weakening of successive generations.

In spite of the many proofs of sexuality in plants, there were still many people who doubted and attacked the theory bitterly.

But Karl Friedrich Gärtner published, in 1849, his "Versuch und Beobachtungen über die Bastardzeugung," a masterly review of the whole subject in all its phases, the result of an investigation which lasted 25 years. This was really the final confirmation of the doctrine of the sexuality of plants so brilliantly initiated by Camerarius.

SHAPES OF POLLEN GRAINS

Francis Bauer.—Returning to the morphology of pollen grains, which we left with the brilliant researches of Grew and Malpighi to trace the development of the sexual theory, we find the next important work to be that of Francis Bauer, the artist. Unfortunately, however, most of Bauer's work was never published and consequently had no effect on the development of the science. Many years after Bauer's death we find investigators rediscovering certain points showing themselves to be in complete ignorance of others which were well known to him. He had seen and drawn well-developed pollen tubes, probably before the close of the eighteenth century, yet Amici is credited with their discovery in 1830. Of course it is far from certain that Bauer understood the function of pollen tubes because he wrote nothing about them. He recognized the extent to which family likenesses are borne by pollen grains and other points which were not cleared up until nearly half a century later. I believe that if Bauer's work could have been published during his lifetime, the science of pollen morphology would now be considerably in advance of what it is today.

The greater part of Bauer's work is bound in a single volume at the British Museum (Natural History) at South Kensington. This work consists of numerous drawings, illustrating more than 175 species, in 120 genera and 57 families. All except a few of these are in the form of pencil sketches in an unfinished condition and with no verbal description. Most of the figures are small and crowded together on sheets of cross-section paper which was obviously used as a scale of dimensions and which he varied to suit the size of the grains being depicted. The whole appearance of the unfinished sketches suggests great haste; only the essential features are recorded and the name, with occasionally a word or two about the color. Nevertheless, the sketches are surprisingly accurate, and the fact that Bauer was generally

able so readily to single out the essential features shows that he had an understanding of pollen morphology far in advance of his time. His method of working appears to have been to sketch the grains as circumstances afforded opportunity, probably in connection with his illustrations of the plants themselves drawn for other purposes, for there is no order in their arrangement. It was apparently his intention to redraw these with all their details at a later date. This was, however, fulfilled in only a very few cases. But there are in the collection a few finished drawings, and these are of great beauty, clarity, and accuracy of detail and frequently colored. They are much larger than the sketches and usually drawn only one to a page.

On account of the inaccessibility and great interest to all students of pollen morphology which attach to the drawings of Bauer's, most of the species represented in his collection in the British Museum have been classified and listed below. The time when Bauer made the drawings is not exactly known, for none bears a date. But the evidence of the few drawings of this kind which were published in connection with his illustrations of plants suggests that the work extended over a long period of time, possibly the greater part of Bauer's stay at Kew, from 1790 until his death in 1840.

List of Pollen Species Sketched by Bauer

Coniferae
Pinus uncinata
nigra
sp.

Araceae
Arum Colocasia

Commelinaceae
Tradescantia sp.

Liliaceae
Lilium tigrinum
bulbiferum
candidum
Asphodelus hirsutus
Brodiaea congesta
Yucca gloriosa

Amaryllidaceae
Hemerocallis caerulea
sp.
Amaryllis sp.

Iridaceae
Sisyrinchium californicum
Tigridia Pavonia

Musaceae
Musa rosacea

Cannaceae
Canna indica

Orchidaceae
Ophrys nyodes
Nidus-avis
apifera
Orchis mascula
maculata
Listera ovata
Epipactus pallens
palustris
Cymbodium aloëfolium
Neottia sp.
Limodorum sp.
Epidendron elegans
Gymnadenia confusa
Cryptostylis sp.

Proteaceae
Banksia speciosa
media
ericaefolia
Cunninghamii
marginata
Dryandra formosa
floribunda
Leucadendron grandiflorum
Protea sp.
Hakea acicularis

Aristolochiaceae

LIST OF POLLEN SPECIES SKETCHED BY BAUER.—(*Continued*)

Aristolochia Sipho

Amaranthaceae
Amaranthus caudatus

Caryophyllaceae
Dianthus barbatus

Nymphaeaceae
Nymphaea advena

Magnoliaceae
Magnolia grandiflora
glauca

Ranunculaceae
Anemone hortensis
Caltha palustris

Berberidaceae
Berberis asiatica

Papaveraceae
Eschscholtzia californica

Fumariaceae
Fumaria parviflora
sp.

Cruciferae
Arabis alpina

Saxifragaceae
Hydrangea hortensis
Ribes sp.

Rosaceae
Geum chiloënse
Agrimonia mexicana

Mimosaceae
Mimosa pudica
Acacia ciliata
marginata
longifolia
sp.

Leguminosae
Lupinus luteus
sp.
Lathyrus odoratus
Erythrina laurifolia
Pisum nigrum

Geraniaceae
Pelargonium sanguinium
inguinalis
sp.
Geranium Robertianum
Oxalis Bowiei

Tropaeolaceae
Tropaeolum majus

Polygalacaee
Polygala sp.

Malvaceae
Althaea rosea
Malva aphila
sylvestris

Hypericaceae
Hypericum calycinum

Violaceae
Viola tricolor
odorata

Passifloriaceae
Passiflora sp.
quadrangularis
minima
lunata
holosericea
Herbertiana
serrata

Thymelaeaceae
Pimelea decussata

Melastomaceae
Melastoma sp.

Begoniaceae
Begonia hirusta
nitida

Combretaceae
Combretum purpureum
sp.

Myrtaceae
Eucalyptus pelisiodora
pulverulenta
sp.
Melaleuca fulgens
pulchella

Onagraceae
Oenothera speciosissima
Lindleyana
densiflora
Clarkia pulchella
acicularia
Fuchsia coccinea
sp.
Epilobium palustre

Umbelliferae
Eryngium alpinum

Ericaceae
Andromeda calyculata
polifolia
empetrifolia
buxifolia
Kalmia angustifolia
Rhododendron dauricum
Ledum palustre
Erica media
discolor
versicolor

Plumbaginaceae
Statice Armeria

Loganiaceae
Logania floribunda

Asclepiadaceae
Periploca graeca

Convolvulaceae
Convolvulus sepium

Polemoniaceae
Cobaea scandens
Gila tricolor

Boraginaceae
Myosotis scorpioides

LIST OF POLLEN SPECIES SKETCHED BY BAUER.—(*Continued*)

Hookeri
sp.

Verbenaceae
Lantana trifolia
Verbena (blue)
Stachytarpheta mutabilis

Labietae
Salvia splendens

Solanaceae
Franciscea calycina

Hopeana
Solanum nigrum
Petunia phoenicea

Scrophulariaceae
Calceolaria
Verbascum virgatum
Antirrhinum majus
Pentstemon sp.

Veronica sp.

Bignoniaceae
Bignonia sp.
Capreolata

Acanthaceae
Thunbergia alata

Dipsaceae
Scabiosa atropurpurea

Cucurbitaceae
Cucumis sativus
Cucurbita sp.

Rubiaceae
Oxyanthus speciosus
Manettia sp.

Lobeliaceae
Lobelia gigantea
sp.

Compositae

Leontodon Taraxacum
Cichorium Intybus
Sonchus hispanicus
palustris
Doronicum sp.
Gnaphalis sp.
Aster fruticulosus
chinensis
Madia elegans
Anthemis Cotula
Achillea Millefolium
Rudbeckia digitata
Dahlia frustranea
Cineraria glauca
Gaillardia bicolor
Cladanthus arabicus
Centaurea cyanus
Seratula quinquefolia
Calendula officinalis
Zinnia elegans
Tagetes sp.

Besides these unpublished pollen figures some of Bauer's published drawings of plants are accompanied by figures of their pollen grains. For example, the *Curtis Botanical Magazine* for 1832 carries, as No. 3172, an illustration by Bauer of an orchid, the large-leaved pterostylis (*Pterostylis Banksii*). It consists of a beautiful colored picture of the whole plant, an enlarged flower, enlarged dissections, and a group of seven pollen grains, six in water and expanded and the seventh apparently dry and in air, consequently not expanded. These grains he found to be quite different from those of other orchidaceous plants. Of them he says:

> I have now, on the second of May, examined the pollen grains with Ploessel's grand microscope, and to my great surprise, have found a total deviation from those of all the hundreds of specimens of orchidaceous plants I have yet investigated. These grains in their ordinary form consist of three- or four-celled corpuscles. . . . This I consider an important circumstance, and could not be detected by botanists possessed only of a glass of moderate power.

In this connection it is interesting to note that in Bauer's British Museum collection are illustrated grains of 15 species of Orchidaceae and that, of these, all except one (*Epipactis pallens*) are represented as four- or occasionally three-celled. So one is at a loss to explain his surprise at finding the grains of pterostylis to be three- or four-celled, unless the figures of the British Museum collection were all drawn subsequently to that of pterostylis, which would require the entire collection to have been done during the last eight years of Bauer's life, which seems improbable. It is true, however, that his figures of the grains of pterostylis do not resemble those of any other orchidaceous plants, nor do they answer his description.

Bauer wrote nothing more about multiple-celled pollen grains, but if one can judge from the mute evidence of his drawings they intrigued him greatly, for among his collection are found far more than the normal proportion of such forms. Furthermore, those which he chose to draw appear not to have been taken entirely at random but selected rather to cover the whole field of such forms as far as possible, and never did he let an unusual form escape him. The 16-celled grains of the acacias in their characteristic geometrical arrangement are shown again and again, but along with them are the exceedingly rare forms of *A. ciliata* with 12 cells and of *A. marginata* with 10. Besides these he showed the 16-celled *Acacia* grains in most of their irregular arrangements. One wonders, in looking over his sketches, if he understood that the normal 16-celled grain possessed its peculiar-looking arrangement because it is made up of four tetrahedral tetrads. It is likely that he did, for his drawings show that he knew that the grains of *Mimosa*, which is closely related to *Acacia*, occur only in fours—always in tetrahedral tetrads. He showed in the four-celled grains of *Epilobium palustre* and in 10 different species of Ericaceae that the grains are always four-celled and that the cells are in the tetrahedral arrangement. In the latter he quite correctly interpreted the furrows, but in neither case does it appear that he understood the spatial relation that the pores of three-pored grains bear to the pores of their three neighbors in such tetrads. Perhaps the microscope which he praised so highly was not quite equal to the task. Or was it because his interest in the multiple-celled grains was occupied with the mathematics of their arrangements to such an extent that other details slipped by him? At any rate, when he came to the four-celled grains of *Periploca graeca* he found and interpreted all the curious arrangements in which these grains occur, but in none

of his numerous drawings of them did he show the germ pores which face each other across the sutures between the adjoining cells. He found that the grains of *Oxyanthus speciosus* are in fours and that those of *Myosotus scorpioides* may be two-celled. With the consideration therefore, that multiple-celled grains are relatively scarce among pollen grains in general, it seems that Bauer must have carefully recorded all such, whenever encountered, on account of an innate interest in the curious. In this he expressed a scientific curiosity scarcely at all appreciated in his day, for his contempories seem to have regarded him always as Francis Bauer the Artist, paying scant heed to Francis Bauer the Scientist.

This is interestingly brought out in the many illustrations which Bauer made in fulfillment of his office of "botanical painter to His Majesty, George III." His floral dissections which accompany these pictures, besides being works of art, are scientifically accurate, and with many of the illustrations he showed the pollen grains as with his pictures of pterostylis. This plan of showing figures of the pollen grains along with each species illustrated was not new with Bauer. It had already been adopted by William von Gleichen in "Das Neueste aus dem Reiche der Pflanzen," in 1764. Gleichen always showed his pollen grains in a circular inset, one-half of each grain drawn in its dry condition and the other in its wet condition, which showed that he understood the difference between the two and the importance of recognizing both. Unfortunately, however, Gleichen's figures were not drawn by himself. It is true that they appear to have been done by an excellent artist, but since, as Sachs says, microscopic drawings cannot pretend to take the place of the object itself but are rather intended to show to another person what passed through the mind of the observer, they fail in their purpose, appearing merely as unnecessary ornaments. With Bauer the case was somewhat different, for throughout the greater part of his career at Kew, Bauer was closely associated with the great English botanist Robert Brown, since they were both subsidized in their work by Sir Joseph Banks. And from this union of an excellent botanist with an inimitable artist of keen botanical understanding were born some notable results. Much of Brown's work was illustrated by Bauer. This is true, for instance, of Brown's memoir "On the Proteaceae of Jussieu," which was published in the *Transactions of the Linnaean Society* in 1809. In speaking of the pollen of this family Brown says, "I am inclined to think, not only from its consideration in this family, but in many others, that it [the pollen] may be consulted with advantage in fixing our notions of limits of genera." And he incorporated in his diagnosis of the family a description of the pollen grains: "pollen triangulare, angulis subsecernentibus, quandoque ellipticum v. lunatum raro sphaericum." The figures by Bauer illustrating the triangular, elliptical, lunate, and spherical forms of pollen grains bear out the diagnosis and testify to the value of pollen-grain forms in classification. And one cannot suppress a sigh of regret that the example here so admirably set by Brown and Bauer has not been followed in other taxonomic work.

It is quite plain that in this case the idea of the importance of pollen characters came from Bauer rather than from Brown. As a matter of fact, in some of Brown's publications the work is almost entirely that of Bauer, the name of Brown, the botanist, merely giving botanical sanction to the work of Bauer, the artist. This is true of Brown's report "An Account of a New Genus of Plants Named *Rafflesia*," which was published by Brown in the *Transactions* for 1820 and consists entirely of the beautiful illustrations, one of them colored, by Bauer. In this, however, the pollen grains are drawn with insufficient magnification to show any detail. Perhaps a better example of Bauer's work is found in Brown's report on the fertilization of orchids and milkweeds, published in the *Transactions of the Linnaean Society* for 1833. The chief value of this paper lies in its truly masterly illustrations by Bauer, which are certainly among the best of this great artist's work now extant. Accompanying those of *Asclepias purpurescens* and *A. phytolaccoides* are figures of the dissected flowers and their pollinia. These latter are shown in their various stages of development, in their ordinary condition of maturity and germinating, both on the stigma and in vitro. His figures of the germinating pollinia with the pollen tubes streaming out, one from each pollen cell, are marvels of beauty and accuracy and would do credit to any textbook of botany today. Brown tells us that when he discovered, in 1830, the significance of the pollinia of the milkweeds, he communicated his findings to Bauer and found that Bauer had already (in 1805) discovered and illustrated the germination of these pollinia

but kindly permitted Brown to use the illustrations. The keen understanding shown in this work shows that Bauer the botanist was the peer of Bauer the artist, who was recognized by all as supreme.

The story of Bauer's life is simply and beautifully told in the epitaph on his tomb in the church at Kew where he lived and did his work. It reads:

> In memory of Francis Bauer, Esq., F. R. S., F. L. S. &c., Botanical Painter to His Majesty George III, and resident draughtsman for fifty years to the Royal Botanic Gardens at Kew, where he devoted himself to the advancement of natural science; under the munificent patronage of Sir Joseph Banks, Bart., the president of the Royal Society. In the delineation of plants he united the accuracy of a profound naturalist with the skill of an accomplished artist, to a degree which has only been equalled by his brother Ferdinand. In microscopical drawing he was altogether unrivalled, and science will be ever indebted for his elaborate illustrations of animal and vegetable structures, of which invaluable specimens are preserved at the British Museum and in the University of Göttingen. He was born at Felsperg in Austria, on the 4th. of October 1758 and accompanied his friend the Baron Joseph Jacquin to England in 1788. He settled at Kew in 1790 where he lived admired, loved and respected. He died on the 11th. of December 1840, aged 82 years. The *works* of Francis Bauer are his best *monument*. Friendship inscribed this record on his honoured tomb.

Before Bauer's death there had already appeared a sudden burst of interest in pollen morphology. Almost every botanist of note was intrigued by it, and his interest held for a little time. This was probably due to the fact that the period at the beginning of the century was marked by great improvement in the microscope. "In 1824 Selligue exhibited to the Academy of Paris an excellent microscope with double lenses, several of which could be screwed on one over the other, with a magnifying power of five hundred times; in 1827 Amici made the first achromatic and aplanatic objectives with three double lenses screwed on one over the other," and it was about this time that the microscopes by Ploessel referred to by Bauer were made. Sachs in his history of botany says, "How rapid the progress was before and after 1830 is shown by comparing von Mohl's work on climbing plants of 1827 and its antiquated illustrations, with his publications of 1831 and 1833, when the figures have a thoroughly modern appearance."

Of the investigators following Bauer a few stand out for their contributions to the morphology of pollen grains; Purkinje, von Mohl, Mirbel, Fritzsche, and Meyen. The pollen works of these were produced more or less simultaneously between the years 1830 and 1839, so that it is difficult for the chronicler to present them in their proper order. The work of all except Purkinje has a decidedly modern aspect. Purkinje, whose work slightly antedates those of the others, appears, therefore, to have been unable to avail himself of the most recent improvements in the microscope. Nevertheless, his observations and interpretations are so far in advance of those of his predecessors that he must needs be credited with a large share in the advances made, though they were so soon to be eclipsed by those of von Mohl and Fritzsche. Purkinje's work on pollen appeared in 1830 while that of von Mohl appeared five years later, and that of Fritzsche in several parts was given to the world 4 to 7 years later.

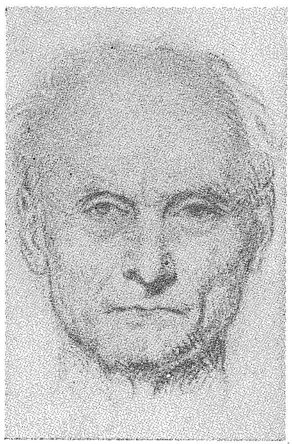

FIG. 6.—Johannes Evangelista Purkinje, by Joseph Mánes. *Reproduced from the Scientific Monthly by kind permission of Dr. Victor Robinson.)*

Johannes Evangelista Purkinje.—On account of his Bohemian birth and isolated position in the scientific world, the works of Purkinje, who was born in 1787 in the village of Libochowitz on the Elbe, are but little known. During his lifetime they did not have the effect upon the general current of scientific thought to which their originality and keen understanding entitled them. Likewise his work on pollen is entirely unaffected by the hot disputes that were being carried on in France, Italy, and England. But he was not born in a peasant hut, as has sometimes been stated. He was born in the castle of Baron Herberstein for whom his father was agricultural official.

The young Purkinje attended school in his native village, going later to the Piarists in Moravia where he studied philology. Upon his graduation he entered the order of the Piarists as a teacher of ancient languages. Later he went to Prague, his native capital, where he occupied himself with literature and teaching, and in 1819 at the age of thirty two he turned to the study of medicine. In this he specialized in physiological optics, graduating with a thesis on the subjective aspects of vision. His thesis was read by Goethe at Weimar, and so impressed was the great German poet with the rare scientific ability of its author that a lasting friendship grew up between the two men, a friendship which shaped the whole subsequent career of Purkinje, for when he applied for the chair of physiology at Breslau it was through the influence of Goethe, coupled with that of Alexander von Humboldt, that he was successful in his candidacy in 1823.

At Breslau he found himself in a decidedly hostile and unsympathetic atmosphere. When he required a microscope the idea was scoffed at. For, why should a physiologist need a microscope? He was forced to carry on his experiments in an unequipped corner of the laboratory, and later he even found it necessary to transfer them to his own home. In the meantime his classes dwindled away. But it was amidst such surroundings that Purkinje did his greatest work. Gradually, however, his exceptional attainments became recognized at Breslau. Students began coming again to his classes, and later the Prussian government erected for him a building devoted exclusively to physiology, which was opened in 1839 as the first Physiological Institute. But these latter years at Breslau were for Purkinje largely sterile of scientific achievement.

In 1850 he returned to Prague as professor of physiology. His return to his native country was celebrated not alone in Prague but throughout the provinces, and the government gave him a splendid laboratory with a capable assistant and adequate salary; here he remained until his death in 1869. But these years, though supplied with everything that he needed, like his latter years at Breslau, were without notable contributions to science. It was amidst the hostile surroundings during earlier days at Breslau and with improvised apparatus that his greatest work was done. His biographer, Dr. Robinson, says, "It is the glory of Purkinje that he holds a foremost place among the investigators who found physiology a speculative subject and left it an experimental science."

Purkinje was primarily a physiologist. His first work was in physiological optics. His method of lighting the retina, his measurements of the lens and cornea, and measurements of the refracting surfaces of the eye made the ophthalmoscope of Helmholtz possible. He studied fingerprints and was the first to recognize their permanent and distinguishing character. He will always be remembered for the Purkinjean nerve cells which form a characteristic feature of the cerebellum and for the Purkinjean fibers of the cardiac muscle. In botany he introduced the terms *cambium* and *protoplasm* and others. And, before Schleiden and Schwan, he taught that organs consist of cells and nuclei. But, like most investigators of first rank, he conducted many minor researches, and among them was his study of pollen grains.

Purkinje's work on pollen is embodied in the single volume "De cellulis antherarum fibrosis nec non de granorum pollinarum formis, commentationis phytotomica," Breslau (1830). The first part of this work is devoted to a profusely illustrated discussion of the microscopic appearances of anther walls, in which he attempted to bring out their specific differences, a subject which appears never to have been adequately studied since. The second part of the book, entitled "De formis granorum pollinis relate ad familias naturales adnota nonulla," begins his discussion of the pollen grains of the same species of which he had studied the microscopic characters of the anthers.

In the preliminary discussion he tells us that the forms of pollen grains may be spherical or triangular and all in between and that these forms are distributed among the most diverse families so that some have one form, some another, and other families both. He then proceeds to characterize the pollen grains of the different families. Grains, simply smooth spheres, he says, are encountered in the Alismaceae and, principally so, in the Gramineae and Juncaceae and many others which he names. Smooth spheres and oblong spheres are found in the aroids, Iridaceae, etc., also the primitive asclepiads. But in the cucurbits and Malvaceae they are hispid spheres. The characteristic forms of many groups are thus given, and when families cannot be so characterized he gives the forms by genera, pointing out that the basic characters of pollen grains may be family or generic. And, while he never attempted to construct a key for identification, as most subsequent investigators have done, in his classification of the characters of pollen grains he

provided the materials for such a key. His recognition of the characters of pollen grains possessing phylogenetic distribution was a decided step in advance of his predecessors.

His descriptions show that he examined his pollen grains in the moist and expanded condition, yet in none of his figures are the grains burst or extruding their contents, as they are in most of those of Bauer. It therefore appears that Purkinje had found a suitable technique for expanding the grains without bursting them, and it is quite evident that he regarded this as the proper condition to reveal their characters. He had at his disposal methods of preparing them in a dry condition had he wished to do so; indeed, he was the discoverer of the use of balsam as a mounting medium, and it is known that in technical ability he was unsurpassed in his time.

Purkinje believed that the various forms of pollen grains were largely the result of their development while in contact with each other in the anther sac. If we look into the beginning of pollen grains, he says, we find that in the anthers while still in the bud they are generally appressed tetrahedral but that when their surrounding pressure is released they expand into spheres, or, if the pressure is longer continued and with less loosening, they keep their tetrahedral form. Compound grains remain in the condition in which they were in the bud. He believed that the positions of both the protuberances and the pores of the grains, which he calls *hila*, are the result of the contact and pressure relations of the grains with each other. The spines, etc., of rough or hispid grains, he states, are merely strongly developed intercellular fibrillae. In his study of the pollen of the passion flower he described the large, operculate pores, stating that the opercula are nothing but the sides of a plane tetrahedron, presumably arising from its tetrahedral contacts with the neighboring grains in the anther. And in other grains which do not have opercula, these contact points, when the grains are torn apart, leave weakened spots through which the inner coat bulges out, forming the familiar germinal papillae. That such is actually the case with the spores of some fungi has recently been shown by B. O. Dodge. And with pollen grains it is true that of those which have their pores or furrows in the trischistoclasic system the system is generally, if indeed not always, initiated by just such contacts with two or three of the neighboring cells of the tetrad. Purkinje's statement is as close to the actual truth as he could possibly have come in the light of the then current cytology. Undoubtedly the whole truth of the situation would have been clear to him had the karyokinesis of the reduction divisions of pollen mother-cells been then understood.

Purkinje made the first attempt to build up a system of nomenclature for the description of pollen grains. His terms generally clearly and accurately described the structures to which they were applied, and many of them are so appropriate that they might be advantageously adopted even today. The germ pores he called hila, apparently borrowing the term from the name denoting the point of attachment of a seed to its pod, having in mind the former attachment which he believed the grains to have had with each other in the anther, for he knew quite well that pollen grains are never attached to their anther sacs in the way that seeds are attached to their placenta. In some ways this term is fully as good as the modern term germ pore, which is faulty because it draws attention to only one function of the pore—a function which only one pore in a grain generally serves—and so directs attention away from an equally important function, that of volume-change accommodation, a function which generally requires more than one pore or furrow in each grain. When the pores are surrounded by a thickening, as in the grains of *Nerium*, Purkinje called them halonate; when borne on horn-like projections, as in the grains of *Oenothera*, he called them corniculate; when sunken in pits, as in the grains of *Tilia*, he called them pariform. The pine grains, with their lateral bladders, he called myocephalic; and the grains of *Saxifraga* he described as meridionate, yet even today writers frequently describe the meridionally arranged furrows of such grains as parallel, which, of course, is self-contradictory when used in connection with a sphere. Such grains as those of the mints, which have banded furrows, he called zonate. The compound grains he described as conglobate, which is perhaps a more appropriate term than the current word compound, for it clearly designates an assemblage of *individuals*. It also has a variety of convenient usages which the other has not; for example, if there were three grains joined together, which he stated to be the case with the Epacridaceae, he described them as triglobate; or if four are joined together, as with the grains of *Bignonia* and *Catalpa*, he

described them as "grana quaterne conglobata." Of the *Acacia* grains he says that there are 6, 7, 8 or, more frequently, 16 "in orbicula conglobata." Purkinje's system of nomenclature deserved much more attention than was ever given to it by subsequent investigators. A system of this kind, had it been put into use, would have saved much confusion, for to give a thing a name is to recognize its existence, thereby directing attention to it—the first and most essential step toward its explanation. These are only a few of his terms, but the fact that he felt the need of them shows that he saw clearly and that the characters of pollen grains and their significance had for him a vivid reality. This we may regard as Purkinje's great contribution to the morphology of pollen grains.

Purkinje's botanical work is curiously detached and self-sufficient, as if he were a lone invader of the botanical field. This, as we have already seen, was due to his geographically isolated position. As a matter of fact, the time when Purkinje's paper on pollen was published was a period of unprecedented botanical activity, and, though we find no contributions of a purely morphological character before von Mohl and Fritzsche in 1832–1837, there were many investigations on pollen which, while centering mainly on the problem of fertilization, had a profound effect upon the development of our science. We must, therefore, digress a moment and examine some of these.

"FERTILIZING" GRANULES

Needham (1740) had discovered that many pollen grains, upon being brought into contact with water, expand, extruding papillae at their pores, and eventually burst, discharging their contents. The contents, consisting of a viscous fluid charged with granules, he believed to be the fertilizing material, supposing that, in nature, when discharged on the stigma, the granules made their way through channels in the style to the ovules. An entirely different view was held by Kölreuter (1761), who believed pollen grains to consist of a cellular core covered by a thin, delicate inner membrane and a tough, elastic outer coat. The bursting of the latter in contact with water he regarded, as we have seen, as unnatural and guarded against in nature by the extreme elasticity of the inner coat, which could bulge through the pores as the grain expanded and so act as safety valves to keep the grain from bursting. The fertilizing substance, he thought, was secreted from the pollen grains in the form of the oily fluid commonly found on the surface and was required to mingle with a similar secretion from the stigma to form a new substance which flowed through the style to the ovule where it initiated the formation of the embryo. This view, of the combining of two substances on the stigma, however, he later retracted, finding that it was irreconcilable with the facts revealed by his experiments in hybridization.

Baron von Gleichen Russworm, in "Das Neueste aus dem Reiche der Pflanzen" (1764), attacked Kölreuter on all points and stated that the granules which Needham had observed pollen grains to discharge, upon being immersed in water, moved like animate bodies. He therefore considered that they were endowed with life and were comparable to animal spermatozoa. He thought that one of these entered the ovule and became the embryo. This was in conformity with the current theory of "evolution" and was really a modification of that theory as put forward by Moreland in 1702, who believed that the pollen grain contained the embryo and that the whole grain must pass from the stigma through a tube in the style to the embryo sac where it became implanted. This theory, as stated later by Christian Wolff (1723), hypothesized the presence of embryos borne by the sap throughout the plant, eventually becoming lodged at the leaf bases to form buds or at the base of the stem to form bulbs—so why not in the pollen grains, eventually to be implanted in the embryo sac, to form seeds? The bursting of the pollen grains with the emission of granules which appeared to be endowed with independent movement, to Gleichen, was evidence that the pollen grain contained not one but many such prospective embryos.

The matter was studied by Turpin (1820), who was the first to draw attention to the formation of the pollen tube. He stated that the pollen wall consisted of two layers which he called the exhymenium and the endhymenium (*exhyménie* and *endhyménie*) and that the fertilizing fluid or fovilla is contained within the latter. When such a pollen grain is placed in water the outer membrane ruptures, and the inner extends, forming a thin-walled intestinal-like tube bearing the fertilizing fluid from the grain. Soon this tube, he said, breaks up, and the fluid is discharged bearing its excessively small granules, which are endowed with independent movement and are comparable to animal spermatozoa. It is not entirely certain if what Turpin saw was really a pollen tube, or if it was just the contents of the pollen grain streaming out into the water and assuming the form of a tube.

To Giovanni Battista Amici, professor of mathematics at Modena in 1824 more properly belongs the credit for the discovery of the *pollen tube*. He appears to have first appreciated its significance, though his remarkable discovery was made quite by accident. He had found that the stigma of *Portulaca oleracea* was covered with hairs which contained granules, and he wanted to see if they moved as he had seen similar granules move in the cells of *Chara;* he found that they did. His statement of the way in which they move is meticulously concise and accurate, contrasting sharply with the statements of most authors of the time, and, therefore, immediately commands respect. Repeating the observation, he accidentally found a hair with a pollen grain attached, and while he was watching the movements of the granules in the hair, the pollen grain suddenly split open and sent out a kind of tube which was somewhat transparent. Growing along the side of the hair, it entered the tissue of the stigma. He described it as a simple tube filled with granules which circulated in and out of the grain. This, he found, continued for nearly three hours, ending in the disappearance of the granules, but he was not able to tell whether they returned to the grain, entered the stigma, or dissolved away bit by bit. He allows the reader to infer, however, that the prolific humor or fertilizing fluid was carried into the stigma by the pollen tube, for he states that Kölreuter and Gärtner believed that the splitting open of a pollen tube is an unnatural process, the result of an excess of moisture, and that the prolific humor, residing on the inside of the inner elastic membrane, filters through only bit by bit. "We have, therefore," Amici says, "observed an exception to their opinion in the pollen of *Portulaca oleracea*." One wonders if he really regarded the behavior of the pollen of *P. oleracea* as an exception or as an example of the rule itself.

In following the story of pollen morphology through this period, one wonders at how firmly the belief in the independent existence of spermatic granules, animalcules, or plant spermatozoa, as they were called, had become entrenched in men's minds. But no less curious were some of the beliefs entertained regarding the structure of the grain itself, as bit by bit its structure became revealed. The two layers of the wall and their remarkable differences from each other had become generally accepted, though Gleichen (1764) and Hedwig still believed that there was only one layer in the wall. As we have seen, Robert Brown, in generalizing the form of the grains of the Proteacea, says that they are triangular with secreting angles, which compensate for the lack of secretion of the stigma.

Guillemin.—It is unfortunate that Brown mistook the germinal papillae, which bulge prominently from the germ pores in these grains, for glands, for the idea was picked up and extended by Jean Baptiste Guillemin (1825), who, in his "Recherches microscopiques sur le pollen," based a classification of pollen on their supposedly secreting organs. He said that pollen grains derive their color from a sticky fluid on the surface, which might be mistaken for an outer coat but may be removed by alcohol, for it is only the product of the papillae, mammillae, and other projections of the outer membrane, which are in reality organs of secretion. Some grains, like those of *Cobaea scandens*, which we now know are coarsely reticulate and covered with an abundance of oil, he described as cellular and having a nipple in each cell secreting the viscous material. This type of grain he consequently called mammillary. He classified pollen grains "according to the presence or absence of secreting organs" as viscous and nonviscous. The former class he divided according to the nature of the supposed secreting organs, whether they were papillae or mammillae. These groups he further divided according to the number and arrangement of the secreting organs. The echinate grains of the Compositae he characterized as papillate; and the lophate grains of the Cichorieae as having facets or flattened mammillae (*mamelons*), taking exception, in this, to the descriptions of Mirbel and Amici who had already more nearly correctly described grains of this character. The latter author, in a short paragraph appended to the story of his observation of the germinating pollen grain of *Portulaca oleracea*, had stated that the grains of *Cichorium Intybus* were spheroidal, with faces corresponding to those of the pentagonal dodecahedron, a

statement which, though not entirely correct, is much closer to an actual expression of fact than that of Guillemin.

Perhaps the most interesting part of Guillemin's paper is his description of the response of pollen grains to moisture, for the phenomenon is but little known and scarcely at all understood even today. He says, "All smooth, nonviscous elliptical pollen grains, in contact with water, absorb this fluid almost instantly (never later than a second) by a suture or longitudinal cleft by which they are marked. A rapid movement of separation is seen in this suture; the grain swells and becomes perfectly spherical." This is an accurate description of what takes place in practically all pollen grains which are provided with germinal furrows, but it is not necessarily confined to nonviscous, smooth grains.

Brongniart.—The remarkable discovery of Amici and the curious conception prevalent at this time regarding the spermatic granules and the secreting glands of pollen grains excited the interest of the young botanist Adolph Brongniart, who undertook to straighten out the problem of fertilization in flowering plants. How comprehensive his study was intended to be may be seen from the following problems which he set himself: (1) the structure and development of pollen; (2) the interaction of pollen and the stigma; (3) the means of communication between the stigma and ovule; (4) the structure of the ovule; (5) the introduction of the fertilizing substance into the ovule; (6) the development of the ovule and its interaction (*rapport*) with its surrounding tissue. His paper was read before the Paris Academy of Sciences in 1826 and published a year later as "Mémoire sur la génération et le développement d'embryon dans les végétaux phanérogamiques" (1827). How successful he was in convincing the academy that he had solved all these problems is seen by the fact that they awarded him the prize for experimental physiology.

He accepted the current conception of a pollen grain as a vesicle filled with fine granules which escape from it when moistened. He was at first unable to decide whether the granules are absorbed by the grain during its development or are generated within but concluded that they are most likely absorbed and that in the grains of *Oenothera* the angles with their bulbous projections are absorbing organs and not, as Brown had said, organs for secreting oil. In fact, he states that all pores and furrows, whatever their form, are organs for absorbing the pollen granules during development and for their emission at maturity. The walls of the grain he described as consisting of two layers, of which the outer is thick and often cellular, as, for example, in those of *Cobaea, Ipomoea, Datura,* and *Mirabilis,* mistaking in these the reticulate surface pattern for cells. He repeated the experiments which Amici had done with the pollen of *Portulaca;* he found that he was able to get pollen tubes in the same way from all kinds of pollen, and he saw clearly that an extension of the inner coat covered the tube as it grew out. He found that some grains, such as those of *Oenothera,* put forth tubes from two or more of their pores. The tubes, he thought, bore the spermatic granules which he considered to be the most important part of the grain and undoubtedly analogous to animal spermatozoa because they appeared to have an independent movement. He found that their movements were slowed down by lowering the temperature, and this, in turn, he considered to be the cause of the inability of some plants to set seed in cold weather. Also, he found that the granules were of different sizes and shapes in different species. He undertook to measure them, in spite of their extremely small size, and furnished tables of their measurements in many different species.

Setting himself to the second problem—the action of pollen on the stigma—he accepted Amici's discovery of the pollen tube penetrating the stigma, at the same time rejecting the theory of Kölreuter, Link, and Gärtner, who supposed that fertilization is accomplished by the slow seepage of a resinous material through the pollen membrane. But where Amici's observations left off, Brongniart's imagination carried on. The whole stigma, he said, is designed to absorb the fertilizing fluid. The stigma is covered with transparent vesicles, the stigmatic hairs, which in most cases are prolongations of the cells or vesicles of which the stigma and style are composed. The pollen tube or spermatic tubule, as he called it, penetrates deeply into the tissue of the stigma, becoming expanded at its distal end. At this stage it may be dissected out. Brongniart's observations and his accurate figures, if they had stopped here, could have constituted an important step toward the solution of the problem of fertilization, though but a slight step beyond Amici; but his lively imagination, ever ready to supply where observation failed, led him to see the pollen tube burst and discharge its vibrating "spermatic granules" among the cells of the stigma and to see them migrate down the whole length of the style and enter the placenta and embryo sac. His illustrations of their progression are quite convincing.

In some kinds of plants, like *Hibiscus* and *Nuphar,* the stigma, he said, is provided with an epidermis which underlies the stigmatic hairs. This epidermis he believed to be a thin homogeneous membrane of a noncellular nature. The pollen grains he found to be caught by the projecting stigmatic hairs which prevent them from coming in contact with the membrane. He said:

> It appears to me that in the case of these, the pollen grain sends out from its interior a prolongation of the inner membrane or spermatic sac; this membranous tube applies itself to the epidermis of the stigma, which is equally membranous, the two uniting at their surfaces, thus establishing a direct communication between the spermatic cavity of the grain and the space beneath the epidermis of the stigma, in the same way as between the tubes of such Algae as *Spirogyra* at the time of conjugation. Thus the spermatic granules pass from the pollen grain into the stigma. The spermatic granules themselves then penetrate the stigma and are really the active part in fertilization.

Brownian Movement.—At about the time when this remarkable paper of Brongniart's was being awarded a prize by the Paris Academy of Sciences, Raspail, in his studies of the structure and development of pollen, concluded that movements of the pollen granules were due to external causes, and that they were not endowed with life. The question of this phenomenon was taken up by Robert Brown (1828), who observed the movements of the granules in the pollen of *Clarkia* and satisfied himself that they were not due to extraneous causes, such as evaporation and convection currents, concluding that the movements were inherent in the particles themselves. Having observed this movement in the pollen of most diverse plants, he sought it among the spores of mosses and *Equisetum* and then in the leaves and other parts of plants, finding it just the same. Then he looked in the pollen from herbarium specimens that had been dried for 20 and 100 years. He thought that in the pollen of the old herbarium specimens the movement was a little slower, but he doubted if the particles could live so long, so he examined oölite fossil wood, which could be burned, and found that when he ground it up fine enough the particles vibrated just like those of pollen grains. Then he examined silicified wood and finally stones which bore no fossils, with the same result. From these experiments he came to the conclusion that the vibration of particles was the property of nearly all material of whatever origin but that it was inherent in the particles themselves and not, as Raspail had asserted, due to extraneous causes. It is from these researches of Brown's that the phenomenon—Brownian movement—bears his name. He did not think that the discovery of the almost universal presence of this property militated against the theory of pollen spermatozoa, though, in the meantime, Raspail had brought further evidence in support of his contention that the vibrations of pollen granules were due to external causes and that the particles were not living animalcules.

Raspail's charges were regarded seriously enough by the Paris Academy to cause them to appoint a committee to investigate the phenomenon. The committee consisted of Cassini, Desfontains, Mirbel, and de Blainville. Its report is interesting. Cassini, after summarizing the statements set forth in the memoirs of Raspail, Brown, and Brongniart, expressed the opinion of the committee as follows:

> Your commissionaires, after devoting themselves to the observation of the facts with all the care of which they are capable, and discarding from their minds all prejudices, have unanimously agreed with M. Brongniart and M. Brown that the extraneous causes to which M. Raspail attributes the movement of the granules do not have anything to do with it.
>
> On the other hand we recognize with M. Brown that various inorganic substances ground in water, offer, if not always, at least sometimes, bodies of which the size, shape and movement are almost the same as those of the pollen granules: such are the outward appearances. But must it necessarily follow that the inner nature, all the properties and functions, are absolutely the same in these bodies of such diverse origins? This we shall not have the temerity to decide, and cannot with assurance until after much more numerous researches and more profound than those which we have made.

Nevertheless, the committee agreed that the pollen granules pass into the stigma and, traversing the style, accomplish fertilization, as described by Brongniart. As a further expression of their faith in the author of the prize essay, they recommended Brongniart for membership in the Academy.

Brongniart immediately responded with another memoir—"Nouvelles recherches sur le pollen et les granules spermatiques des végétaux" (1828)—reasserting all his claims and stating that pollen granules differ from those of lifeless material in that the former not only move but also change their shape as they do so. Having settled this question to his own satisfaction and to that of the Paris Academy, he made one of the most surprising statements:

> By the action of water or the humidity of the stigma the external membrane [of the pollen grain] *contracts* and pushes out the inner membrane, which emerges as a projection through the pores by which the outer membrane is pierced, causing the formation of one or more tubes which finally burst, setting free the granules which penetrate into the stigma.

It is difficult to understand how Brongniart could have thought that pollen grains behaved in such an anomalous fashion when moistened, for their sudden *expansion* under this condition is one of their most striking characteristics, one that can easily be seen even with low magnification and which had been adequately described by Guillemin three years earlier.

On account of the sanction and acclaim that Brongniart's work received from the Academy, it came to have a profound influence upon the development of the science. In the same year we find Mayen, in his "Anatomische-physiologische Untersuchungen über den Inhalt der Pflanzen-zellen (1828)," making essentially the same statements regarding pollen spermatozoa, and, as we shall see later, even the careful Hugo von Mohl was not entirely immune to Brongniart's influence, though he, from the first, vigorously denied the spermatozoon-like character of the pollen granules.

Amici.—It appears, however, that Amici was not convinced, for we find him two years later (1830) writing a note "sur le mode d'action du pollen sur le stigmate" to Mirbel on the same subject. He says that one can see the circulation of the prolific humor in the pollen tubes of many grains besides that of *Portulaca* in which he had made his original observation, but that it is seen best in the grains of *Hibiscus syriacus*. In these he saw generally

2 or 3 tubes coming out of the grain at the same time and in other species of Malvaceae as many as 20 or 30 from a single grain. He states that the tube or tubes, as the case may be, emerge from the grain and penetrate into the stigma.

> This is quite certain and can be shown with many plants: but is the prolific humor passed out in the interstices of the conducting tissue as M. Brongniart saw and drew it, to be next transported right to the ovule, as supposed by that author? No; it is the tube which elongates bit by bit, the whole length of the style and comes in contact with the ovule, to each ovule corresponds a tube.

He says that, of course, the pollen grain could not provide enough nourishment for the tube to traverse the length of the style; therefore it must receive its nourishment from the tissues of the style; but his caution prevented him from committing himself on this point for which he had only indirect evidence. However, he observed that the pollen granules traverse the tube and continue to circulate all the time.

In these few words Amici contributed more toward the development of our science than possibly all other investigators of his period put together. If we are to draw a lesson from this, it is surely that one careful observation, accurately recorded, is worth more than numerous argumentative discussions and that the offering of a prize is not a good way to uncover the truth in a controversial matter.

While these researches were being directed to the discovery of the structure and function of pollen grains, but little was done that was strictly morphological, that is, of a comparative nature, or in the way of furnishing material for a comparative morphology. Kölreuter (1761) had pointed out that plants which are related ordinarily have similar pollen but that as much similarity may sometimes occur among the pollen of plants which are only very distantly related. Brown (1809) set an excellent example in his taxonomic discussions of the Proteaceae by including descriptions of the pollen-grain forms in his diagnoses. Sprengel (1812) announced for the first time that the pollen grains of many dicotyledons have three furrows, a fact the recognition of which is as important in morphology as the discovery by Malpighi of the single furrow among the grains of most monocotyledons. Amici, as we have seen, in 1824 correctly interpreted the form of the grain of *Cichorium Intybus*, describing the faces as corresponding to those of a pentagonal dodecahedron, which, in effect, they do, except that their arrangement is disturbed by the presence of three germ pores. The apparently faceted nature of the grains of the Cichorieae had already been noted by Mirbel, but he had failed to recognize the geometrical relation of the facets to each other. In this respect Amici had a decided advantage in that he was a profound mathematician. Guillemin, in 1825, believed that the forms of pollen grains could be as useful in the classification of plants as the seeds which had been used by Gärtner and Richard. But Guillemin's attempt to classify pollen-grain forms on the ill-conceived basis of the nature and function of what he took to be their secreting glands did not substantiate his contention. Of greater moment was his accurate description of the vicin threads (*fils visqueux*) which he found on the grains of *Oenothera*, the curiously interesting, delicate threads which hold the pollen grains of *Oenothera* and many Ericaceae, etc., together in a cobwebby mass. They have to this day not been satisfactorily explained.

Mirbel.—The next important advance in morphology is that of Charles François Brisseau-Mirbel, in 1833, in his studies of the pollen of *Cucurbita Pepo*, *Hyoscyamus alba*, *Cobaea scandens*, *Passiflora brasiliensis*, and *Lilium superbum*. This work is but little known, because it is appended to his much larger work on *Marchantia polymorpha*. Yet it is the most exhaustive and accurate study of its kind that had been made up to that time. He treated the development of the pollen mother-cells of *Cucurbita* from their first anlaga, and the formation of the tetrads, which must be regarded as the most important advance in this direction since 1820, when Brown and Bauer in their study of *Rafflesia* had discovered that pollen grains were developed in special cells and not just in the anther cavity, as formerly believed and asserted by Turpin in the same year. But of still greater importance were Mirbel's descriptions and figures of the mature grains, for these are accurate and at the same time interpret the meaning of the structures represented. Mirbel had been an artist but was early introduced to the study of botany by Desfontaines, with the result that we find in him that rare combination of artist and scientist which we have already seen produced such remarkable results in Bauer and seems to be essential to the development of a morphological science in its formative stages. The value of correct drawings is generally greatly underestimated, many people even assuming that good photographs must be more reliable. In making microscopical drawings the eye is compelled to dwell upon the contours and minute detail of the object. At first these appear to be without arrangement, but after the eye has studied them and put them accurately together on paper an underlying plan is revealed, and from the apparent chaos gradually emerges the plan of the whole which may then be resolved into its structural elements. These may be captured and retained only as dictated to the mind by the trained eye and skillful hand. As more forms are examined the same structural elements are found to occur again and again in different combinations, and different but related plans are revealed. In this way the morphological science is built up as a visual structure. It is only after the units of this structure have become fixed by repeated examination and recording and become accepted by others that they may be given names. At this stage which is relatively far along in the development of a science, and scarcely yet attained in the development of the morphology of pollen grains, a system of nomenclature may gradually take over a part, at least, of the burden of drawings. Mirbel had already, in his "Anatomie végétale" (1802), attempted such a system of nomenclature by furnishing a glossary of descriptive pollen terms, but this was destined to fail because it had outrun his knowledge of pollen structures. And we find him, in his description of the *Cucurbita* pollen grain, making little use of his own glossary, relying, instead, upon his truly superb drawings. These show the grains unexpanded, expanded with the germinal papillae protruding, and with the wall ruptured so as to display its inner and outer coats.

Classifications Based on Pollen Characters.—The first successful use of pollen characters in classification appears to be that of John Lindley in 1830, in his genera and species of orchidaceous plants. In these the pollen grains are always shed united in masses of varying sizes, shapes, and degrees of compactness. The pollen masses are webby in appearance in some species and waxy in others. When the latter, they have a sticky gland whereby they may become attached to the bodies of insects. In some species the sticky gland is borne on a long stalk or

caudicle, in some on a short stalk, and in others the gland is unstalked. These characters form the basis for the establishment of four tribes in Lindley's monograph. Julius Fritzsche, in his "Beiträge zur Kenntniss des Pollen" (1833), showed for the first time the possibility of classifying pollen-grain forms extending over a large number of families. But his classification, while fairly comprehensive, is entirely artificial. He divided all forms of pollen grains into those with single grains and those with compound grains, which at once splits such families as the Onagarceae, Typhaceae, and Mimosaceae into two, because in each case it is only some of their members which have compound grains. His next divisions were those with furrows and those without. That Fritzsche made no attempt to make his classification approximate the natural system seems evident from this, for the presence of a single furrow in the pollen grains of most monocotyledons and of three in most of the dicotyledons had already been noticed by several authors. This work of Fritzsche's must be regarded as only introductory to his much larger work "Ueber den Pollen," which appeared 4 years later, after the great work of von Mohl's "Ueber den Bau und die Formen der Pollenkörner," which followed closely after Fritzsche's first paper and covered the entire field, giving to pollen morphology, for the first time, the dignity of a science.

Von Mohl.—Born in Stuttgart, Apr. 8, 1805, Hugo von Mohl came of an illustrious family. His father, Ferdinand von Mohl, was a man of great ability and activity and at different times held important political offices. His mother, a daughter of the Finance Minister of Würtemberg, was an accomplished woman, and to her the young Hugo was indebted for much of his early education. He had three brothers—Robert, who became prominent in government service; Julius, an oriental scholar; and Moritz, a political economist.

For 12 years the young Hugo received formal education at the Stuttgart gymnasium, specializing in classical languages; but while there he occupied his leisure in the study of botany and mineralogy, guided somewhat by the botanist Frölich, and in the study of mathematics, especially optics, in which he became remarkably proficient. In 1823 he went to Tübingen to study medicine, graduating in 1828. His father wanted him to adopt medicine as a profession, but he, preferring botany, prevailed upon his father to let him go to Munich where he furthered his botanical studies in the society of Schrank, von Martius, Zaccharini, and Steinheil, devoting himself mainly to a study of the anatomy of palms, ferns, and cycads. In 1832 he became professor of physiology at Bern, and it was while there (1834), at the age of twenty-nine, that he published his, "Über den Bau

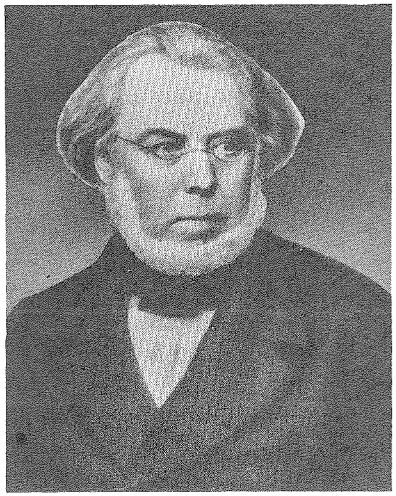

FIG. 7.—Hugo von Mohl, after the portrait by Cederquist.

und die Formen der Pollenkörner," his only work devoted entirely to the study of pollen.

In 1835 he returned to Tübingen as professor of botany, succeeding Schübler, where he remained practically continuously till the end of his life, excepting the year 1843 when on account of illness he made a protracted stay in the southern Tirol and in Italy which restored him to health. His constitution was generally rugged and vigorous; he frequently made long collecting trips on foot and was seldom troubled by ill health, except toward the end of his life. He never married. He lived a lonely life but was a cheerful and congenial companion to those who knew him well. He was learned in literature and the arts, except music, for which he had a decided aversion. He died suddenly at Tübingen in 1872 in his sixty-seventh year.

There is an excellent short biography of von Mohl by de Bary in the *Botanische Zeitung*,* with a complete bibliography includ-

* **1872**: 561.

ing 90 citations. This has been translated, slightly abridged and omitting the bibliography, in the *Proceedings of the Royal Society of London*.* To this I am largely indebted for the above short sketch.

Von Mohl belongs to the richest period of botanical history and lived in an atmosphere of botanical investigation. He was contemporary with Unger, Schleiden, Hoffmeister, Pringsheim, Nägeli, and others equally illustrious. And it is hard to imagine the development of botany during this period without the firmly laid foundations of von Mohl. It has been said that "to give anything like a full account of von Mohl's writings would be to write a history of vegetable physiology." Yet he never composed a connected account of his subject and published only two books, his "Micrographia" (an introduction to the knowledge and use of the microscope) and the "Vegetable Cell." "His efforts as a writer were confined to monographs usually connected with questions of the day or suggested by the state of the literature. In these he collected all that had been published on some point, examined it critically, and ended by getting at the heart of the question which he then endeavored to answer from his own observations. He was usually satisfied with establishing separate facts and in his conclusions kept as closely as possible to what he had actually seen. In scattered monographs of this kind he treated conclusively all the more important questions of phytotomy. Much of his work is found in the *Botanische Zeitung*, which, with Schlectendahl, he founded in 1843 and in which he maintained a constant and active interest throughout his career.

Though his works dealt principally with the solid framework of plants, they were mainly based upon the cell as the unit of structure. He was the first to take up the view that vessels of wood are made up of cells, having observed for the first time their formation from rows of closed cells. And he was the first to appreciate the significance of the plasma membrane, which he called the "primordial utricle," recognizing, however, that it was of the same substance as the rest of the mucilaginous mass which enclosed the nucleus. He later gave to it the name "protoplasm," adopting Purkinje's name for the formative substance within the eggs of animals and cells of embryos, but von Mohl defined it, distinguishing it from the cell sap, and

* **23**: 61–64, 1875.

showed that it was the substance which contained the chloroplasts and carried out the circulation which had long been observed in plant cells.

On the basis of the structure of cell walls, to which the greater part of von Mohl's work was directed, he worked out a classification of tissues, distinguishing for the first time wood, bast, vascular bundles, etc., thus making possible a comparative anatomy of tissues. The more important of his monographs on these various subjects have been collected and published as "Vermischte Schriften."

All through his career von Mohl was much interested in the microscope and microtechnique. He was able to polish and set lenses, and the modern microscope owes much to him for his inventions and improvements of methods for making microscopical measurements. In his "Micrographia" he gave many practical hints to opticians on the construction of the microscope. No less important were his contributions to microtechnique. It was the custom of his time to crush, tear, or macerate the objects for microscopic observation, but von Mohl introduced the preparation of thin transverse and longitudinal sections. In drawing from the microscope he despised the finished drawing which purports to be an accurate and detailed representation of the object. All his drawings he made himself, contrary to the prevailing custom, and in them he showed only the points under discussion, striving merely to make them expressions of his opinions; in his later works, having gained greater facility and clarity in verbal description, he almost entirely dispensed with illustrations.

The greater part of von Mohl's work on pollen was published as "Über den Bau und die Formen der Pollenkörner" at Bern in 1834, among his earliest works. This publication is now extremely rare and hard to come by, so I have had to content myself with the French extract by Leret (1835). The extract, however, is virtually a complete translation, except that the historical part is abridged and the six plates of the original are reduced to three.

When von Mohl set himself the task of bringing order out of the chaos that existed in men's minds regarding pollen grains, he began by critically reviewing most of the work that had already been written on the subject. Much of this he discredited, but

it cannot be said that his pollen work was entirely uninfluenced by current beliefs and theories, though in his later works he gained a notorious independence of thought. It should be said, however, that it was only in his conception of the cellular structure of the pollen wall that he shows any such bias. In this he is a composite of his predecessors, and for this he has been severely criticized by later botanists who came to treat his whole pollen work with scorn, overlooking the great wealth of material that it contains aside from this curious misconception.

Von Mohl approached the study of the morphology of pollen grains imbued with the idea that the outer wall was of a cellular structure. This viewpoint was apparently partly gained from analogy with tissues which he had already been able to prove to be of a cellular nature in most cases. Moreover, Meyen (1828) and others had already stated that the outer coats of such grains as those of *Hemerocallis* and *Amaryllis* were made up of a large number of flattened oil-filled cells. In reality these grains are merely reticulate, the "cells" being the spaces between a system of net-like thickenings which anastomose over the surface of the grain. This bias of von Mohl is one of the very few instances when he allowed a preconceived notion to befog his ordinarily keen powers of intellect and observation. He stated that the cells which make up the outer coat of such grains are five-, six-, or seven-angled like those of ordinary epidermis but very much smaller than any tissue cells. He saw that the dissepiments between them were single-walled, unlike those of tissue cells each of which has its own wall so that the separating partitions between them are double. He found that the walls of these supposed cells were generally thin and smooth and pointed out that they often present the appearance of a network of vessels anastomosing over the surface of the grain and that they were mistaken for such by Kölreuter and Hedwig. But, he pointed out, they do not always have such an appearance, for in the rather exceptional grains of *Cobaea scandens* the walls are thick and appear to be composed of perpendicular fibers. Whatever their form or size, these cells, von Mohl stated, are always filled with a yellow or red oil which he said is not found in noncellular pollen grains. In this von Mohl was obviously following the current opinion, but it appears that his observations did not always bear out this statement, for he goes on to point out that those grains which are not obviously cellular may be as oily as the others but that they are generally more or less granular, though the granules are often so small that they can scarcely be seen even with the best microscope. These granules, he reasoned, must be in reality minute cells, because the grains have oil, and the oily material is formed and retained in the outer wall and is not secreted on to it by glands or papillae, as asserted by Brown to be the case among the grains of the Proteaceae. Indeed, in some grains, like those of *Pitcairnia latifolia*, he found an actual transition between what appeared to be an obviously cellular condition at the equator to a granular condition at the poles. The granules, he thought, must, therefore, be the same as the cells, only smaller.

Following the same reasoning and finding transitions from granular walls to more finely granular, even to completely homogeneous walls, in such grains as those of *Araucaria*, *Rumex*, the Boraginaceae, and the Gramineae, he concluded that in the walls of such grains the cells have so nearly disappeared that the walls present only little obscure points which, though they can scarcely be seen, must exist because the walls have a faint yellow color due to oil which could be produced only by such cells.

Though the outer wall of such grains may often look like the wall of an ordinary plant cell [he says], the comparison of the external membrane with that of a vegetable cell is entirely inexact, and one should regard it as an organ composed of cells or rudiments of cells and a homogeneous element which unites them and, for that reason, compare it not with the simple membrane of a tissue cell but with such compound membranes, as, for example, the membrane of the ovule.

Carrying on this reasoning, he fell into the error which he had condemned in Brown and tells us that the appendages of the external membrane, such as the spines and papillae, are really excessively developed cells which appear to exude oil at their points in the same way that glandular cells do on other parts of the plant and that that is the reason why spiny and papillate grains are generally more oily than those which are smooth. But he correctly points out that this is not a universal correlation, as supposed by Guillemin, for some grains are spiny and have little oil, while others lacking spines have an abundance of oil, and, strange as it may seem, he attributes Guillemin's failure to observe this to his failure to recognize the cellular nature of the membrane in some grains and its transition through granular to almost smooth in others. Von Mohl returns to this theme again and again, repeatedly asserting that "the cells, spines and granules of the external membrane ought to be considered as secretory organs and reservoirs of viscous oil." The idea permeates his whole conception of pollen structure. In a less gifted observer it could have rendered his whole work valueless, but in von Mohl it was relatively much less serious. Sachs says of von Mohl, "He generally connected his researches into structural relations with physiological questions; but . . . he never forgot that the interpretations of visible structure must not be disturbed by physiological views. . . . He used his thorough physiological knowledge chiefly to give a more definite direction to his anatomical researches." Never did he let a preconceived notion disturb his vision, as, for example, did Brongniart. Pollen morphology is a descriptive science, so it is much less serious to misinterpret what is seen than to see awry what has previously been misinterpreted, provided that the observations are correctly and accurately reported. Remove this curious misconception from von Mohl's pollen work, and we have a reliable and accurate guide which could serve us today in almost all other particulars, so it will pay us to pursue the work further.

Regarding the pores, he says, in the grains of some species they occur one in each furrow. In grains without furrows they occur sometimes on the equator, sometimes regularly and sometimes irregularly arranged. As for the shape of the pores, he points out that they are usually round; but those that are situated on the equator are often elongate. "Are these pores real openings," he says, "or are they thinner spots in the membrane like the pores of cellular tissue? This is a question that I cannot answer for the smallest pores, but when they are larger I have been able to convince myself, by separating the membranes, that they are not real openings but are closed by a membrane which is generally thin." In some species he found the pore membrane to be thicker even than the external membrane of the rest of the grain. Such, for example, is the case with the grains of squash, passionflower, and others in which the pore is really closed by a little lid which is pushed to one side or up on top of the tube as it emerges. Often the pore is surrounded by a halo, he points out, which may be less distinctly granular than the rest of the grain. The halo is generally round, but in the grains in which the pores are hidden in the furrows the pore may be surrounded by an elliptical halo, transversely arranged and often longer than the width of the furrow.

Regarding the origin of pollen, von Mohl tells us that the grains are borne in cells, nearly always four in each. "One sees the granular contents of the cells parted into four little masses; in consequence of development, these masses are replaced by four grains of pollen which adhere more or less strongly to each other. Later the grains separate and, finally when the cells which contained them have disappeared, remain free in the cavity of the anther." He also noticed that the arrangement of the four cells was sometimes according to the four angles of a tetrahedron and sometimes all in one plane; in the dicotyledons tetrahedral in all species which he had observed, but in the monocotyledons he found them arranged both ways. Though this is not the whole story, it is a remarkable advance over Brown's discovery in 1820 that pollen grains were formed in special cells.

The greater part of von Mohl's study of pollen grains is presented in the form of a descriptive classification of their forms. I have reproduced this here in an abbreviated form, because, though written 100 years ago, it still has value and has never been really superseded.

Von Mohl's Classification

A. Coat of a single membrane—Asclepiads.
B. Coat of two membranes
- † Furrows and pores absent
  - i. Surface granular—*Calla, Crocus, Sagittaria, Ranunculus*, etc.
  - ii. Surface papillate—*Canna, Gaertnera paniculata, Bauhinia*, etc.
  - iii. Surface cellular—*Phlox, Ruellia, Aleurites*, etc.
    - *Derivative forms*—grains in fours
      - *a.* Tetrads in one plane—*Apocynum, Vellosia, Periploca.*
      - *b.* Tetrads tetrahedral—*Juncus, Luzula.*
- †† Longitudinal furrows present
  - A. Furrow one (most Monocotyledons)
    - I. Surface granular, without adornments
      - *a.* Furrow membranes (*Streifen*) granular—many Monocotyledons and *Myristica.*
      - *b.* Furrow membranes smooth—Monocotyledons, *Ginkgo, Liriodendron*, and *Magnolia.*
    - II. Surface granular, with spines—*Nymphaea alba, N. advena*

    - III. Surface cellular—Hemerocallis and other Monocotyledons
    - IV. Surface reticulate—*Alstroemeria.*
      - *Derivative forms*, grains united in fours—Orchids.
  - B. Furrows two—*Cypripedium, Amaryllis, Calceolus, Pontederia cordata, Tamus, Dioscorea, Tigridia, Watsonia, Micranthus, Calycanthus* (rare and always flat with the furrows on the edges).
  - C. Furrows three, longitudinal
    - I. Surface granular, furrow membranes granular—Several Dicotyledons.
    - II. Surface granular, furrow membranes smooth—*Lacis fucoides, Balanophoreae*, Cynomorium. *Nelumbo* and many other Dicotyledons, the commonest form.
  - D. Furrows more than three
    - I. Furrows four—*Houstonia, Sideritis, Cedrela, Platonia, Blackwellia*, and many others which are normally three-furrowed.
    - II. Furrows six—Some Labaetae, Ephedra, *Sanguisorba*, Passiflores.
    - III. Furrows many—Rubiaceae, *Penaea, Sesamum.*
      - *Derivative forms:*—(*a*) form of *Pinus* (intermediate between the one-furrowed Monocots and the three-furrowed Dicotyledons). (*b*) Form of *Lotus.* (*c*) Form of *Poinciana.* (*d*) Pollen prismatic—*Tropeolum majus, Ximensia.* (*e*) form of *Loranthus.* (*f*) Polyhedral, such as *Corydalis, Fumaria, Clerodendron, Rivina.* (*g*) Spiral:—*Thunbergia, Mimulus, Hypericum.*
- ††† Outer coat with pores
  - *Primitive forms:*—
  - A. One pore—Gramineae, Cyperaceae, *Typha angustifolia, Spargainum, Restio, Cecropia, Anona.*
  - B. Two pores—*Colchicum, Broussonetia* and several Monocotyledons and Dicotyledons.
  - C. Three pores
    - I. Surface granular—*Oenothera*, Amentiferae, Urticaceae and other Dicotyledons.
    - II. Surface cellular—many Passiflores.
  - D. Four pores
    - I. Pores on the equator—*Myriophyllum, Boehmeria, Campanula rotundifolia, Trigonia*, etc.
    - II. Pores not on the equator, *Passiflora, Impatiens, Balsamina.*
  - E. More than four pores
    - † Pores distributed regularly
      - (*a*) On the equator—*Alnus, Betula, Ulmus, Goniocarpus, Campanula, Thryallis* (in the last more or less towards one pole).
      - (*b*) Over all the surface—*Basella alba*, etc.
    - †† Pores scattered irregularly

(a) Outer coat granular, smooth—Nyctaginaceae, Thymeliaceae, Convolvulaceae, Chenopodiaceae, *Alisma Plantago, Celtis australis, Plantago lanceolata.*

(b) Outer coat granular and spiny.—Malvacelae, *Cucurbita, Ipomoea.*

(c) Outer coat cellular.—*Polygonum orientale, Persicaria, Cobaea.*

Derivative forms (*i.e.*, of †††, outer coat with pores)

(a) Grains in tetrahedral tetrads, *Jussieua erecta, Drimys Winteri* etc.

(c) Form of Mimosaceae.—*Inga, Acacia* etc.

†††† Outer coat with longitudinal furrows and pores

Primitive forms

A. Round with three pits and three pores.—Dipsicaceae, Geraniaceae.

B. With three furrows and three pores

(a) Outer coat granular—Common in Dicotyledons.

(b) Outer coat with spines—Most Compositae.

(c) Outer coat cellular—*Turnera, Grewia, Stackhousia, Syringa, Ligustrum, Celastrus.*

C. Outer coat with more than three furrows, each with a pore.—Boraginaceae, Polygalaceae etc. and exceptions among the three-furrowed forms.

D. Six to 9 furrows, of which three contain one pore each *Vinca rosea.*

E. Three or four furrows, and three pores not in the furrows.—*Carolinea campestris, C. longiflora, Eriodendron.*

Derivative forms

(a) Tetrahedral tetrads.—Epacridaceae, Vacciniaceae.

(b) Cubic and dodecahedral forms.—Malpighiaceae—*e.g., Gaudichaudia.*

(b) Polyhedral with three pores and three furrows *Lactuca, Vernonia montevidensis* (transition form).

F. Wall of three membranes.—Some conifers, *e.g., Taxus.*

It is evident that von Mohl recognized the furrows of the grains to be their most important morphological features, a fact which has often been overlooked by later investigators, particularly those who insist upon examining pollen in its dried and shrunken form. He points out that the arrangements of the furrows are enormously varied, though they generally are meridionally extended. But, whatever their arrangement, they are always so formed that the folded part projects inwardly when the grain is dry, "but when the grains are made wet they expand and the part of the furrow which was hidden becomes a part of the external surface of the grain . . . and the part which was hidden in the folds always presents a different structure from the rest of the outer membrane although it is in immediate continuity

with it." In their expanded form he calls the furrows *Streifen*, and states that they are generally transparent, though they may be granular, but if so the granules are quite different from those of the rest of the outer membrane and may have characteristic arrangements in different species.

Von Mohl recognized for the first time the geometrical configurations that the furrows assume and their importance to his classification. He noticed that three furrows arranged meridionally was the commonest condition among the dicotyledons but that there are also many other numbers and arrangements among them. He found that in the grains of *Corydalis lutea*, for example, there were six furrows. The surface of these grains, he said, is found to be divided by six fissures into four triangles, or, in other words, the furrows correspond to the six edges of a tetrahedron. Other grains of this species he found to have nine furrows, corresponding to the edges of a triangular prism with its sides rounded out. And in the grains of two species of *Rivina* and one of *Fumaria* the furrows are so arranged that they divide the surface of the grain into pentagons, forming of it a pentagonal dodecahedron. In some species he found that a single furrow configuration was characteristic but that in others two or more configurations could be found even in the pollen from a single anther. Passing to the spiral forms, he found that he could establish a sequence of forms ranging from that with three furrows meridionally arranged to the strictly spiral forms, such as those found in the pollen of *Thunbergia* and *Mimulus* in which a single furrow follows a zigzag course over the surface of the grain. He therefore concluded that the spiral forms were derivatives of the ordinary three-furrowed configuration. His analysis of these furrow configurations was truly a remarkable feat, expressive of his great genius for interpreting what he saw. In his interpretation of *Pinus* pollen, however, he was somewhat less successful. He regarded it as a derivative from the one-furrowed form of the monocotyledons but at the same time intermediate between it and the three-furrowed form of the dicotyledons. The single longitudinal furrow which occupies the ventral side in the grain of *Pinus* he correctly interpreted as the functional furrow, but the two re-entrant angles that the bladders make with the cap at their dorsal roots (Fig. 78) he interpreted as additional rudimentary furrows, homologous with those of the three-furrowed dicotyledons. Apparently, in this grain he saw a connecting link between the monocotyledonous and the dicotyledonous form of grain.

The echinolophate forms of the Cichorieae he described as "polyhedric," with three pores and three furrows, approximating in form a pentagonal dodecahedron. And the pollen of *Vernonia montevidensis*, in which the lophate form is only partially expressed, he pointed out as intermediate between the polyhedric and the more usual echinate form of the Compositae.

A highly suggestive part of von Mohl's work is the portion containing his descriptions of the pollen forms by families; to give an example: "Gramineae, oval, glistening, nonviscous, outer coat finely granular, not separable from the inner; on one side a punctiform umbilicus with a small halo." In families where more than a single form was found: "Chenopodiaceae (*a*) cubic with blunt edges, in the middle of each face a nongranular part like a pore, *Basella alba*, (*b*) spherical, outer coat finely granular, provided with about twenty pores, *Blitum capitatum*, *Salsola scorparia*."

In this way he characterized the pollen of 211 families with the formal brevity of taxonomic diagnoses. It is a matter of interest that the genus *Basella*, regarded by the older taxonomists as belonging to the Chenopodiaceae—von Mohl states that he followed Bartling's "Ordines plantarum"—is now separated from the Chenopodiaceae and put in a family of its own. Nor is this an isolated example. As we read through von Mohl's pollen descriptions by families we encounter many violent breaks in the succession of forms. Some of these have since been smoothed out by improvements in the classification; others remain and stand today as challenges to taxonomy. The succinct way in which von Mohl put forth his descriptions suggests that he felt that they formed a part of the family or generic descriptions. At any rate, he presented them in a form which could easily have been taken over and put into the family diagnoses, but they have to this day been ignored, in spite of the enormous wealth of material which they present with their obvious implications.

Two years after the publication of this paper by von Mohl, Fritzsche read before the St. Petersburg Academy of Science his "Ueber den Pollen," pointing out the errors in von Mohl's conception of pollen structures. Even though von Mohl never actually retracted his views, they became greatly modified in later years. In his "Vegetabilischer Zelle" (1851, page 123), for example he says:

> The perfect pollen grain consists of a cell . . . covered on the outside by a membranous layer which owes its origin to a secretion, and, in particular cases, is separable into two or three superincumbent layers. The outermost layer, corresponding to a cuticle, is mostly rather tough, uniform, or covered with granules, spinules, projecting linear and often reticulated ridges, mostly colored and the seat of a more or less abundant secretion of a viscid oil.

Apart from this von Mohl seems never to have referred to the matter again.

It may have been the appearance of Fritzsche's immortal work, seeming to knock the theoretical foundations from under von Mohl's, which prevented him from returning to the subject, causing him to leave it in this unfinished condition. Whether or not this supposition is correct, the fact remains that, after immersing himself in the study of pollen morphology for many years and amassing enormous quantities of material in one of the richest fields of botany, von Mohl abruptly left it and busied himself with other subjects which seem to us distinctly less interesting. It is also probable that von Mohl's work, antedating that of Fritzsche by a little over a year, anticipating all his main results, had a similar effect on Fritzsche, for, as we shall see, he also dropped pollen morphology, never to return to it, after he had put into print his "Ueber den Pollen," thereafter directing his attention entirely to chemistry. It is seemingly to this circumstance that we still have the field of pollen morphology largely unworked before us; because either one of these great minds could have made such inroads upon it and would have provided such an enormous stimulus to further investigation that pollen morphology would by this time have advanced to a stage of completeness comparable to that of the other branches of science to which these two men lent the powers of their intellects.

Carl Julius Fritzsche.—Born on Oct. 29, 1808, in Neustadt, in Saxony, Fritzsche was the son of Christian Ferdinand Fritzsche, medical officer in charge of public health of Stolpen and

Hohenstein. His mother came from the family of Struve. Both parents lived until the year 1833 to see their illustrious son given the degree of Doctor of Philosophy in Berlin.

In Neustadt, where Fritzsche spent his childhood, there was no school, so up to his fourteenth year he received private instruction directed toward the study of pharmacy; and later he went for the same purpose to Dresden, where he was employed for five years in his uncle Struve's pharmacy. From Dresden he went to Berlin, where he superintended for 2½ years the laboratory of the pharmacy of Helming. But finding this occupation clearly not scientific, he took the assistantship at the chemical laboratory of the famous chemist Eilhardt Mitscherlich, and this was the most important event in Fritzsche's educational career.

Mitscherlich had been a student of Link and Berzelius and had distinguished himself as the discoverer of dimorphism in crystals and of benzene sulphonic acid and nitrobenzene. Besides his accomplishments as a scientist, he had published part of a history of Persia in Latin and Persian. Thus it happened that Fritzsche's close association with Mitscherlich was of great benefit to him. The master shared his knowledge freely with the young man, and in this position which he held for 2½ years he found in every nook and corner an unfolding of his leaning toward the natural sciences. Fritzsche reports this association with Mitscherlich as his happiest recollection, and from his "Curriculum vitae" we fully understand the feeling that bound him to his master. In this he expresses himself in these words, "In this time I hold the greatest affection for Mitscherlich. With the deepest gratitude will I remember him to my grave. With fatherly care he guided my work and made it possible for me to round out my knowledge."

It was probably due to Mitscherlich's influence that Fritzsche entered the faculty of philosophy in the university in 1831. He records in his *Vita* that there he attended the lectures of Mitscherlich and such other distinguished men as the two Roses, chemist and mineralogist; Lichtenstein, the zoologist; and Kunth, the botanist. In 1832 he published his "Beiträge zur Kenntniss des Pollen" and in 1833 took his degree of Doctor of Philosophy with "De plantarum polline" as his inaugural dissertation. In 1834 he published "Ueber den Pollen der Pflanzen und das Pollenin" in Poggendorf's *Annalen*.

In the same year he moved to St. Petersburg where he became a member of the St. Petersburg Academy of Science, and his great work "Ueber den Pollen," read before the academy in 1836, was published in the "Mémoires des savants étrangers" of the Academy in the following year. After that time his publications, which are numerous—over 60 are recorded—dealt almost entirely with purely chemical subjects, principally organic. Among them we find treatises on uric acid, the anhydrides of potassium nitrate and nitrous acid, alkaloids, indigo derivatives, carbohydrates, and many others. Most of his papers dealt with fundamentals, and some of them constituted milestones in the progress of science, as, for instance, his discovery that murexides were ammonium salts of purpuric acid, the decomposition of anthranilic acid into aniline and carbon dioxide, the discovery of the isomers of nitrophenol, and others. Such achievements as these required patience, industry, and precision of observation. These attributes Fritzsche possessed to the highest degree; in fact, he has been criticized for his excessive attention to details. It was this character, however, which led him safely past the pitfalls into which von Mohl had stumbled in his morphology of pollen.

Much of Fritzsche's time was commandeered for purposes outside his beloved sciences. In 1848 he was made chief of the commission for the study and organization of the Caucasian mineral waters, of which he was conducting the analyses at the time. He was chemist of the medical department and advisory member of the medical council to the Minister of the Interior; also, he took part in many commissions, as that on household gas illumination, the commission for the introduction of electric illumination into Russia, and the building of St. Isaac's Cathedral. He had now become Staatsrath Fritzsche. But all his free time he spent in his laboratory; at first he had only a small one near his home, but later moved to the new and spacious chemical laboratory of the Academy which he directed in association with N. Zinn.

The home life of Fritzsche was on the whole peaceful and happy, but he was twice a widower, and after the death of his second wife he was left for 15 years with the care and responsibility of bringing up his son and daughter. His relations with his colleagues were of the happiest character. Often they searched him out in his laboratory in friendly visit, eagerly expecting to hear from him something new and strange, also because they regarded him as a man of amiable character and good heart, ever active in the accomplishment of good.

Up to his final sickness he enjoyed excellent health, but in the year 1869 he suffered an apoplectic stroke which made of him a crippled old man. Although he recovered somewhat from it, he remained lame, and speech and memory had almost left him.

FIG. 8.—Karl Julius Fritzsche. (*Courtesy of the Soviet Academy of Sciences, Leningrad.*)

The friends who had known him when he was hale and hearty were grieved to see him in these latter days; he himself preferred death to such a life. For only a very short time did he resume his scientific activity—long enough to finish his study of the molecular changes of tin.

In the following year he journeyed to his native land in search of physical and mental relief. The latter he found in the circle of his family, who eagerly welcomed him home. His illness, however, became steadily worse and on June 20, 1871, brought him the desired release from his sufferings. Nevertheless, he was happy on his deathbed, knowing that he had not lived in vain and that his life had enriched those with whom he had come in contact as well as the science which he loved. Truly it has been said that "the name of Fritzsche is indelibly written in the annals of learning next to the facts with which he enriched Science."*

Fritzsche's Works.—Before the publication of von Mohl's work on pollen in 1834, Fritzsche as we have seen, had already published his "Beiträge zur Kenntniss des Pollen" and his "De plantarum polline," and in the same year as von Mohl's paper, his "Ueber den Pollen der Pflanzen und das Pollenin" was published in Poggendorf's *Annalen*. Strictly speaking, therefore, he deserves priority over von Mohl, but these works were only preliminary to his principal one "Ueber den Pollen," which was published in 1837, 3 years after von Mohl's.

* The facts of Fritzche's life are derived from A. Butlerow (1872) and from the Curriculum vitae in "De plantarum polline" (1833).

In his "Beiträge" Fritzsche pointed out that the recent improvements in the microscope called for further advances in the study of pollen. The instrument he used was by Pistor and Schick, which he regarded as even better than the very fine instruments by Amici, Chevalier, or Ploessel. With this microscope he was able to get a magnification higher than necessary, or even desirable for the study of pollen grains, and the high resolving power and brilliancy of illumination of this microscope, which are really more important than magnification, are abundantly attested by his studies with it of starch grains, in which he shows the concentric rings quite clearly.

Having such an instrument, Fritzsche stated that it was necessary only to devise appropriate methods of preparing the pollen to reveal their characters much more fully than had ever been done before. A large part of the Beiträge is taken up with a discussion of the various methods of expanding pollen grains and rendering them transparent, which he recognized as essential to their study. Of the different methods, he selected treatment with varying concentrations of acids as the best adapted to the purpose.

With deftness and precision he defined the problems in hand to be whether the pollen of a species is of an invariable form; whether the different species of a genus agree in their pollen forms as they do in their other characters; what rule governs the distribution of the different pollen forms in the natural system; and whether the more highly organized dicotyledons also have more highly organized pollen.

From a preliminary survey of his material he at once came to the conclusion that the different forms of pollen grain, while more or less constant within the species, do not correspond to the natural classification, nor do the more primitive plants have the more primitive forms of pollen. But, he concluded, a classification of pollen forms has just as much right and may be just as natural as the classification of the plants themselves. Accordingly, he laid out a classification in some respects superior to that of von Mohl. He took as his primary divisions, for simple grains, (1) those with and (2) those without furrows. Of the former he made eight subdivisions, according to the number of their furrows: (*a*) grains with 1 furrow, *e.g.*, *Amaryllis;* (*b*) with 2 furrows, only *Justicia adhatoda;* (*c*) with 3 furrows, the commonest form among the dicotyledons; (*d*) with 4 furrows, generally occurring in pollen of species which normally have other numbers of furrows; (*e*) with 6 furrows (in this group he includes only those with their furrows meridionally arranged); (*f*) with 8 furrows, *e.g.*, *Symphytum officinale*, with a structure similar to that of the 3-furrowed forms; (*g*) with 10 furrows, *e.g.*, *Penaea mucronata* and *Asperula taurina;* (*h*) with 20 furrows, *e.g.*, *Polygala latifolia*, of which the furrows are meridionally arranged and only 4 have germ pores.

The grains without furrows he subdivided into those with and those without pores. Those with pores he subdivided again into those with one pore—only the grasses—and those with several pores. The latter are again subdivided into those with pores arranged in a circle around the grain, *e.g.*, *Banksia* and *Sida;* and those with the pores equally distributed—smooth, *Ribes;* with spines, Malvaceae.

The compound grains he treated as a separate group, subdividing it into (1) 4 grains united, without pores, *e.g.*, *Luzula;* (2) 4 grains, each with three pores, *e.g.*, Ericaceae; and (3) 16 grains united, *e.g.*, *Acacia*.

This is only a brief outline of his classification but will serve to show how complete was his understanding of pollen. One cannot but be struck with the similarity between this classification and that of von Mohl, yet there is no reason to believe that either author was influenced by the other. The classifications of both probably had the germ of their origins in Purkinje.

"De plantarum polline," which Fritzsche presented in 1833 as his thesis in fulfillment of the requirements for the degree of Doctor of Philosophy to the Friedrich-Wilhelms University, offers little that is new; it is little more than a summary, in Latin and without illustrations, of the work already presented in the Beiträge. His "Theses defendae" are: (1) Pollen granules are nothing but droplets of oil and starch grains; (2) a blue color is effected in starch by iodine owing to a union resulting from a strong affinity between the two substances; (3) grains of starch consist of a gummy material, formed in several concentric layers of homogeneous substances, the whole enveloped by a membrane.

Fritzsche's next paper "Ueber den Pollen der Pflanzen und das Pollenin" (1834) is directed toward the elucidation of the nature and origin of *Pollenin*. This is a substance which may be extracted from pollen by water, alcohol, ether, or alkali. Upon evaporation of the extract a residue remains in which crystals of various form are found, showing that *Pollenin* is not a single substance but a mixture of several. He pointed out that neither the external morphology nor the contents of the grains are greatly changed by the removal of *Pollenin*.

In order to clarify this latter statement a large part of the paper is devoted to the examination of the internal and external morphology. The contents of pollen grains, he stated, consist of three substances: (1) *Schleim*, which occurs in a half-fluid state, becomes dispersed in water without dissolving, is coagulated by weak acid, and is stained brown with iodine; (2) an oil-like substance which is distributed as very fine droplets throughout the whole *Schleim* mass. The droplets do not stain with iodine and can be made to run together into larger masses; (3) small starch grains which may not always be present but may easily be recognized because they turn blue with iodine.

The external morphology of many grains is described in great detail, and Fritzsche pointed out that some of them are strikingly similar to certain crystal forms. For example, the pollen grains of the Cichorieae are six-sided prisms and rhombohedra, and the grains of many Caryophyllaceae are pentagonal dodecahedra. But whatever the nature of the contents or the form of the grains may be, when the pollen is extracted with any of the reagents mentioned above, the grains remain unchanged, except for the removal of the soluble material on the surface. This then must be the source of *Pollenin*. Its chemical nature, however, Fritzsche was not able to discover beyond the fact that it is a mixture of several substances.

In this paper we see the trend of Fritzsche's mind toward chemistry. We also feel that he had at his command a large fund of knowledge of pollen structures. His knowledge of all the principal pollen forms of the Cichorieae and of the Caryophyllaceae, which enabled him to make the statements that he did about their crystal-like forms, suggests that this was the case. As a matter of fact, at this time he knew much more about pollen than he had ever written. The three papers which we have mentioned above told merely of side lights or interesting laws which had come out incidental to his main work, the heroic task of a complete analysis of the pollen forms of all the known plants. We can imagine him carefully building up the structure of his science, block by block, and making each secure before adding the next. It is likely that it would have been many years before the whole structure assumed a form to his liking, so meticulous was he and critical of his own work. But this was abruptly changed by the appearance in 1834 of von Mohl's "Ueber den Bau und die Formen der Pollenkörner," for Fritzsche found in it that von Mohl had anticipated most of his work. So he put together his own results and presented them to the St. Petersburg Academy of Science in 1836.

In "Ueber den Pollen," which was Fritzsche's answer to von Mohl, he stated that he published his results as much to confirm those of von Mohl as for their own worth. But some of his results, he found, were quite different from those of von Mohl, and, besides that, the field recently opened up by the new developments of the microscope he considered so vast that a detailed knowledge at all commensurate with the number of known plants could be achieved only by the co-operation of many workers, whom he hoped that his work would encourage to enter the field. It so happens, however, that Fritzsche set such a high standard of accuracy and keenness of understanding that few have been able to follow him, and no one has ever approached him in the beauty and accuracy of his drawings.

Regarding the contents of pollen grains—the *Schleim*, oil, and starch already referred to—only the last was known chemically; the other two, he stated, are mixtures. But the *Schleim* has the property of absorbing water and swelling enormously. It is this property of the *Schleim*, he said, which causes the grain to crack open when the extent of the swelling exceeds the elasticity of the wall. The contents then become extruded in the water, owing to a contraction of the grain due to the elasticity of the wall upon the release of pressure. The extruded *Schleim* may be stained brown with iodine or coagulated with weak acids. But if the grains have once been dried, the *Schleim* loses much of its capacity for absorbing water.

The oil drops and starch grains, he pointed out, as did von Mohl, make up the so-called pollen granules, and their movements, as shown by Brown, are of a molecular nature and common to all small bodies, though they were mistaken for animalcules. But, he said, it is only the starch grains which could thus deceive, for the oil occurs in spheroidal droplets or fluid masses, while the starch grains are of almost as many different forms as there are kinds of plants; they may be any shape but always turn intensely blue with iodine. It is through their turning endwise, crosswise, and getting out of focus that they give the appearance of changing their shape. Besides, he said, infusoria do not take the least bit of color with iodine; instead it is fatal to the existence of all lower forms of life. In these words he effectively disposed of the fetish of the independent existence of pollen granules.

Intine and Exine.—In discussing the integuments of pollen grains, Fritzsche pointed out that the majority have two coats. A few have one, in which case the outer is lacking; a few have more than two, in which case one or the other is doubled. Therefore, he considered, these coats should be named. "The ending *-ine* seems to me to be the most convenient, as chosen by Mirbel for the integuments of the egg, for it permits itself to be easily modified into German, Latin or French. I therefore name the inner pollen wall the *intine* and the outer the *exine*. When these layers are doubled they may be named accordingly as *exintine* and *intexine*." As Fritzsche had foreseen, the terms have been widely adopted, and we cannot now speak of pollen in any language without using them.

The intine he found to be always the same, consisting of a closed hyaline, colorless membrane which takes no color with iodine and is destroyed by strong sulphuric acid. It is the essential layer; when only one is present it is always the intine. The exine, on the other hand, though not essential and playing no part in fertilization, is complex and enormously various, and it is by it alone that we are able to distinguish the different species of pollen. It is not destroyed by concentrated sulphuric acid, merely turning purplish-red in this reagent.

The foundation of the exine, Fritzsche pointed out, is always a simple membrane and not, as von Mohl had stated, a cellular layer. He went to enormous pains to establish this point. He found that, by treating the grains with sulphuric acid, to dissolve out the contents and the intine, and rolling them between glass plates, he was able to isolate small pieces of the exine, and in some case he actually isolated the granules of the exine. From these experiments he came to the conclusion that the basic structure of the exine consists of a homogeneous matrix in which granules are embedded. These may be of various sizes and shapes, spaced closely together or far apart, or they may be entirely absent. They may be in rows which anastomose, forming a network over the surface of the grain, in which case the homogeneous material may be absent between the rows, nevertheless binding the palisade-like granules together in their rows. A striking example of this he showed in the grains of *Ruellia formosa*, in which the surface is heavily reticulate and presents a decidedly cellular appearance. This is likewise true of the grains of *Eranthemum strictum*, but here in the middle of each space of the net stands a spine or group of spines.

The question of the nature of the spines was next taken up. By the same process as before Fritzsche succeeded in isolating single spines from the exine of a grain of *Ipomoea* and others and came to the conclusion that the spines are the highest development of the granules. Von Mohl had considered that the granules and spines of the exine were reservoirs and secretory organs for oil, but Fritzsche demonstrated by numerous experiments that in no case were they reservoirs for oil; but in the spines he was nearly always able to see a dark streak, like a central core, which he concluded must be an oil canal. "For," said he, "the flowing out of an oily liquid from the majority of different kinds of pollen species through contact with water, which takes place especially strongly with those provided with spines, necessitates passages for this oil, since we cannot believe that it simply adheres to the outside of the grain." It should be stated, however, that the dark central cores which Fritzsche saw in the larger spines were probably merely the result of diffraction of light brought about by the convergence of the rays in passing through the highly refractive material of the spines.

Fritzsche stated that the relation of granules to spines is brought out in the grains of *Ipomoea purpurea*. Here the surface is covered with a network of rows of palisade-like granules, inclosing angular spaces on the surface of the grain, giving it a cellular appearance; and wherever the rows come together there is always a spine which seems to contain a canal.

The spines of the Cichorieae are, he said, the most remarkable of all, but unfortunately he found them so small and fragile that he was not able to isolate them and only once was he able to secure a section vertically through a spine. Of this he furnished a picture, as he said, for what it may be worth, fearful himself to draw conclusions from a single observation. These spines, he wrote, stand in single rows, united by a binding material, and the rows anastomose in such a way as to mark off the surface of the grain into a regular pattern resembling certain crystal forms. Observing the spine rows in side view he was able to see running up into each the same dark streaks which he took for canals.

As an example of a grain which was supposed to have a cellular exine he took that of *Cobaea scandens*. No one has before or since so accurately described this type of grain or figured it half so perfectly. Here, he said, walls stand up on the outer membrane, enclosing on it five- or six-angled spaces all over the surface of the grain. These walls are broken through and have the appearance of a bridge resting on piers. There is no membrane over the spaces or across the gaps in the vertical walls, as there would have to be if they actually enclosed cells, as von Mohl supposed. Furthermore, he continued, the walls cannot without tearing it be separated from the membrane upon which they rest. "It appears to me, therefore, that their nature can only be explained as thickenings of the membrane, which, after they have grown to a certain height, begin to form gaps at their bases."

Although such a pattern is widespread and always suggestive of a cellular structure, Fritzsche said, "An organization of a kind that is truly cellular, I have nowhere been able to perceive with certainty in the exine." And, although this point cannot be regarded as settled for all kinds of grains, he had little hope of ever finding a grain with an exine of a cellular structure.

Turning to such grains as those of *Gilia tricolor* and *Collomia grandiflora*, he described them as having a wickerwork exine, provided with regularly arranged pores. These, he stated, were not admitted by von Mohl to be true openings but were regarded as merely thin spots in the exine. He believed that von Mohl was led to misinterpret these openings by the presence below each of a small body which Fritzsche called *Zwischenkörper*, acting as a plug to close the hole from the inside. It was probably these, Fritzsche thought, which led von Mohl to believe that the germ pores of these and most other grains were not true openings.

The *Zwischenkörper* form the subject of an extensive investigation by Fritzsche. He found that they were hyalin, mostly lens-shaped bodies lying one beneath each pore between the exine and intine. When the grain is moistened they protrude through the pore in the form of a bubble. By rolling and crushing the grains and treating them with suitable reagents he was able to isolate them from the grains of *Astrapaea*, *Ruellia*, *Sida*, and others. In the grains of the Malvaceae, which have numerous pores, the *Zwischenkörper* could not be isolated, but he was able to remove a piece of the exine and showed that under each pore lay a small *Zwischenkörper*. And he concluded that, though they could not always be demonstrated, they must be present in all pored grains.

Fritzsche's Classification.—The second part of Fritzsche's book deals with the different forms of grains throughout the flowering plants. He stated that it had already been sufficiently shown from previous researches that no law governs the distribution of the forms of pollen in the natural classification of plants, nor does the form of the pollen grain always bear any relation to the lower or higher rank that we have assigned to it. But he believed that one could attain a natural classification of pollen forms if one began with the simplest and sought always the next most complex. Such a statement seems strange to us; today if we failed to see any parallelism between the pollen forms and the relationships of the plants we should naturally assume that the fault lay in our inability to interpret the pollen forms or possibly in the classification of the plants. But Fritzsche's idea that two systems of classification running contrary to each other could both be natural shows that he had no appreciation of the significance of the classification of plants. To him it was just a convenient arrangement of forms, as one might classify crystals by the number of their faces, their interfacial angles, their cleavage planes, or however one wished. This was probably because Fritzsche was primarily a chemist; and before Darwin's time the idea of a natural classification among living things was not the underlying principle of all biological studies, as it is today.

Accordingly, he followed the classification of pollen forms already laid down in his "Beiträge." He began his system with the compound grains: "For," he says, "the compound pollen masses of the Asclepiads and Orchids stand indisputably at a lower stage, in all respects, than free grains." The compound grains of *Inga* he included here, but he regarded them as somewhat higher than those of the orchids and asclepiads because they have two coats while the latter have only one.

The major divisions of the single-grained group, he believed, should be drawn according to the number of coats that they have. In Group I, pollen with only one coat, are included *Caulinia fragilis*, *Zannichellia pedunculata*, and *Zostera marina*. *Zostera* pollen he found to be the most exceptional among all pollen, occurring in long, filamentous tubes occasionally branched and filled with granular fovilla which circulates in the tubes, just as Amici had seen it do in pollen tubes. Indeed, he concludes, the filamentous pollen grains correspond to the pollen tubes of most other plants.

In Group II, pollen with two coats, are included the great majority of known forms. Throughout the group, Fritzsche stated, the fovilla and intine are virtually uniform, the enormous variation in the forms of the grains having to do exclusively with the exine. The simplest and, therefore, the most primitive grains, according to Fritzsche, are those without structural openings, *e.g.*, those of *Ruppia*. Then he says, "Next follows a form very widespread among the monocotyledons which consists of an ellipsoid with a longitudinal strip of exine which is naked, representing a longitudinal furrow." In discussing this form of grain he stated that in much of the pollen of *Lilium candidum* he found grains of this character united in pairs, back to back with the furrows facing outward. In the pollen of *Phillydrum* he found similar grains united in fours, likewise with their furrows facing outward, but in the pollen of *Annona tripetala* he found four grains united face to face, *i.e.*, with the furrows inward. When these latter are separated from each other it is true that their contact faces are found to be thin-walled, as would be expected, but they do not possess the form of structural furrows.

It is doubtful, therefore, if Fritzsche was justified in assuming that they were true furrows. On the contrary, it appears to be invariably true that furrows are situated on the outer side of one-furrowed grains in relation to their tetrads. Fritzsche appears to have just missed the significance of this fact.

Continuing with his classified descriptions of pollen, he described grains with spiral bands from several species of *Thunbergia*, but he failed to relate these forms with those with three furrows, as had von Mohl.

Fritzsche's study of the pollen of the various species of passion-flower, however, is much superior to that of von Mohl and has never been equaled since. He recognized the pores at once for what they are, large germ pores, each covered with a lid of the same material and structure as the exine. He described in the minutest detail grains from this genus with three, four, and six pores and grains with three, four, and other numbers of furrows in a variety of arrangements. His beautiful figures of these must be seen to be appreciated.

Furrow Configurations.—Fritzsche fully understood the various furrow configurations. He recognized three meridionally arranged furrows as the basic arrangement among dicotyledons. Six-furrowed grains he found in the pollen of *Corydalis*, and he pointed out that the six furrows were arranged according to the six edges of a tetrahedron. The grains of *Corydalis* are spherical, smooth-walled, and transparent, and the furrows may be seen with great clarity. In the grains of *Basella alba*, however, Fritzsche also found six furrows, but here the grains are cubical in shape and exceedingly thick-walled and rather opaque, with a short furrow arranged diagonally on each of the six faces of the cube. In spite of this, Fritzsche recognized this furrow configuration as also corresponding to the edges of a tetrahedron and analogous to that of *Corydalis*, whereas von Mohl had failed to recognize this, relating, instead, the furrows to the faces of a cube. In the grains of *Talinum patens* Fritzsche found 12 furrows corresponding in configuration to the 12 edges of a cube, and in those of *Polygonum amphibium* he found 30 furrows. Here they are very short—little more than elliptical pores—and the grain is heavily reticulate with high vertical ridges, yet he was able to relate these furrows in their spacial orientation to the 30 edges of a pentagonal dodecahedron.

The grass grains, he showed, have one pore surrounded by a marked thickening of the exine. When dry the grains are cone-shaped, but when made wet they become spherical, with the fovilla protruding in the form of a bubble from the pore, ultimately bursting and discharging into the water, owing to the presence of a *Zwischenkörper*, which Fritzsche did not see but assumed must be present. If he had not made this assumption but had hunted out the *Zwischenkörper*—if, indeed, it may be called that—he would have found that it lies not between the intine and exine but in the body of the grain, generally remote from the pore. When made wet it expands, rapidly filling the entire cavity of the grain, thrusting the other contents out through the pore.

He found that the grains of a great many species have three furrows, with a pore in each furrow. When dry such grains are generally elliptical, and the pores cannot be seen, and, if made transparent by placing them in oil, a complicated internal structure is seen which is due to the marginal thickenings of the inturned furrows and the rims of the pores. This is the condition in most fossil grains, and Fritzsche's figures of grains in oil resemble closely those found in fossil studies. When made wet, however, these elliptical grains expand and become spherical, and the furrows with all their attendant structures may easily be seen.

Pollen-grain Patterns.—In the Compositae, Fritzsche recognized that the basic form was a spiny sphere with three furrows and a pore in each; but in those of *Centaurea*, he found that the spines were so small that they could be seen only with great difficulty. Among the Cichorieae he picked out all the different patterns and described their faces or units which make up the patterns.

Pine pollen, which had so confused von Mohl, Fritzsche examined both wet and dry and after the removal of the exine. He came to the conclusion that its internal structure was the same as that of *Larix*, though the latter possessed no bladders and presented an entirely different appearance superficially. The walls of both, he said, are three-layered, owing to a doubling of the intine. In *Pinus* the exintine is separated from the intine on both sides of the furrow and puffed out to form the two bladders. This, I believe, is the first nearly correct interpretation of the bladders of the grains of *Pinus*.

In reviewing this work of Fritzsche I have mentioned only a few of his statements, selected because they seem to be of special interest, frequently because they have to do with things which von Mohl had failed to notice or had interpreted differently. It is true that when Fritzsche differed from von Mohl, Fritzsche was more often correct, but for the most part the two authors agree, and their work together constitutes the most brilliant chapter in the history of pollen morphology. The two investigators approached the same subject with entirely different intellectual backgrounds. Von Mohl had just discovered that the tracheids of plants were modified cells and he had classified the tissues of stems and satisfied himself that they were all cellular and that the entire plant body was of a cellular structure. What more natural than that he should conclude that pollen grains were likewise cellular structures and regard the reticulate exines, which often have a decidedly cellular appearance, as a confirmation of this view? Fritzsche, on the other hand, was not primarily a botanist and had no preconceived notions of what any of the structures should be, but he had an extraordinary capacity for taking pains. He was much more interested in making observations than in drawing conclusions from them, whereas the reverse is true of von Mohl. The result is that Fritzsche's observations and his extraordinarily beautiful figures are more accurate and more reliable than those of von Mohl, but his reports of them are often wearisome, for in them we miss the brilliancy and purposefulness which characterize von Mohl. In fact, these two authors—Fritzsche, the plodding, painstaking observer, always accurate; and von Mohl, the brilliant theorist, always with a purpose—were by nature and training complementary to each other. It is with a sigh of regret that we must leave them now, for the paths of these two great investigators diverged in the earliest years of their productive period, Fritzsche to become one of the great chemists of his time and von Mohl certainly the greatest botanist of his.

Later Botanists.—The period following von Mohl and Fritzsche up to 1890 when Fischer's "Beiträge" was published, saw the attention of many young botanists directed to the study of pollen. In fact, nearly every botanist and writer on botanical subjects, under the stimulus supplied by the two preceding investigators, referred to the subject in some way; but most of them either quoted the earlier works without adding anything new, as if the final chapter had been written, or else they investigated on their own initiative, heedless of their predecessors, often reporting observations wrongly that had been adequately dealt with by Purkinje, von Mohl, or Fritzsche. The English writers offended most in this direction, possibly because the stimulus from von Mohl and Fritzsche failed to reach across the Channel. On the Continent, however, the period saw considerable advancement; but most of the best investigations of this period were directed mainly toward the elucidation of the development of pollen which had been made possible by the improved methods of technique, and to this the advances that were made in pure morphology were only secondary.

The situation at this time was very well summarized by Franz Julius Ferdinand Meyen, in his "Pflanzenphysiologie" (1839). He himself contributed but little that was new beyond the assertion of doubtful value that most pollen grains possessed a third coat between the intine and exine, which covered the pollen tube during its emergence from the grain. But his work served to clarify the situation by reconciling or correcting to some extent the opposing views which were then extant, and it became the main source of material, and possibly the best available, for subsequent students until the publication of the very much superior work of Hermann Schacht 20 years later.

In order to throw some new light upon the curious pollen structures which had recently become known, Karl Wilhelm Nägeli (1842) undertook to discover the mode of their origins and development. "For," said he, "the correct understanding of a thing depends not only upon a knowledge of its completed form but also of its beginning." He therefore studied the origin and development of the pollen of *Lilium*, *Tradescantia*, *Cucurbita*, *Oenothera*, and *Althaea*. He found that in each loculus of the anther was differentiated a vertical chain of primordial cells which became the pollen mother-cells, through the nucleus (cytoblast) of each becoming resorbed and the development of thickened gelatinous layers on the inner face of the cell membrane. From this stage he found that there were two possible courses of development. In one of these two granular masses are formed in each cell, each with a cytoblast, and at the same time a transitory cell wall is formed; then each cytoblast builds on its outer surface a gelatinous membrane; these he called the primary special mother-cells; then the cytoblast of each cell becomes resorbed, its granular contents divide again into two parts each with a cytoblast, and around each of these arises a gelatinous membrane (secondary special mother-cell). Or, the other possibility, the granular contents of the mother-cell divide into four masses each with a cytoblast and connected with each other by "sap streaming." Around each cell arises a gelatinous membrane (special mother-cell).

This remarkable description has the advantage for us that it almost ignores the nuclear changes. Apparently his material was not stained in a way to render any but the fully organized nucleus visible, so the picture was not confused by the complicated phenomenon of karyokinesis upon which most subsequent investigators have focused their entire attention. To Nägeli, when the nuclear material formed into chromosomes, it seemed to be simply resorbed, and this left his attention free for the observation of other things, such as the arrangement of the newly formed cells and their connection with each other by phragmoplasts, which he called sap streaming (*Saftströmung*).

But, to continue with Nägeli's story, he tells us that within each of the four special mother-cells a pollen cell is formed by the building of a wall (intine) which shuts in its whole contents. Then, through an osmotic excretion, there form on the outside of the pollen cell one or two layers of a substance which is at first gelatinous but later solid and colored (exine), with peculiar modifications in certain places where the pollen tubes grow out (*Zwischenkörper*). Then, the mother-cell and special mother-cells become resorbed, and a viscous material appears which accumulates on the outer surface of the pollen grains.

In the monocotyledons, he says, the formation of the special mother-cells by two successive divisions is the most usual course of development, while in the dicotyledons the four daughter cytoblasts generally form their four special mother-cells simultaneously. This course of development, he says, generally results in a tetrahedral arrangement, but in *Althaea* both the tetrahedral and squared arrangements are found and all in between. This observation is of the utmost importance to the pollen morphologist, since, as we shall see later, the arrangement of the grains in their tetrads determines to a large extent the

arrangement and number of the pores or furrows of the completed grain.

Theodor Weimel (1850) showed that in *Fuchsia* each pollen mother-cell may give rise to two to five pollen grains in all possible arrangements, even four cells in a chain. He also noticed that the mature pollen grains of *Fuchsia* had two, three, four, and six germ pores, but he failed to observe the connection between the two phenomena.

A further advance was made in the same direction by Pringsheim (1854). He showed that in the tetrad formation of *Althaea rosea* after the four daughter nuclei are formed, they may assume a tetrahedral arrangement, or, less frequently, they may be all in one plane arranged either as a rhombohedron or as a square. But in either case, he said, there are weakly developed walls thrown across separating the four nuclei almost as in ordinary cell division, but before the walls are completed they are resorbed and replaced by ingrowths of the gelatinous mother-cell which finally separates the whole into four special mother-cells. Precisely how this is done, however, Pringsheim did not explain, and it was not fully elucidated until Farr (1916) worked out the process described by him as simultaneous quadripartition by furrowing.

The whole pollen situation was excellently summarized again in 1860 by Schacht in his "Ueber den Bau einiger Pollenkörner." This work, compared with the earlier summary of Meyen, shows the strides that had been made during the intervening 20 years. But, besides summarizing and correctly evaluating the previous work, Schacht contributed some important new points. He stated, at the outset, that the grains contain a fluid material consisting of a cell nucleus and insoluble material such as starch grains, inulin, oil, and protein compounds, as well as soluble substances such as sugar and dextrin but no fertilizing bodies. The oil, he stated, is uncolored and not the same as that which is found on the outside of the grain. He described the quadripartition of the pollen mother-cells in mistletoe, essentially as Pringsheim had done for *Althaea*. After the young pollen grains are formed, one in each of the special mother-cells, he showed that three thin spots appear on the outer wall of each while it is still smooth. Soon small spines begin to form all over, except on the three thin spots where the wall does not increase in thickness.

While this is happening the mother-cell and special mother-cells are resorbed, after which the pollen grains lie free without filling the space so that they do not hinder each other in their development. This observation of Schacht's shows us clearly why it is that pollen grains do not have three flattened sides, as commonly found in fern spores, resulting from their being pressed against their three neighbors of the tetrad. It appears, however, that Schacht did not notice that the three thin places mark the three last points of contact that each grain made with its three neighbors of the tetrad.

At this time, as we have already seen, it was commonly believed that the oil which clings to the outside of pollen grains, giving them their yellow color, is a secretion from the interior of the grain. But Schacht found that such oil was entirely absent from the granular contents. "Accordingly," said he, "if it is a secretion of the latter it must be formed while passing through the membrane of the grain," which he rightly regarded as *reductio ad absurdum*, but he did not tell us where the oil comes from.

In the descriptive part of his work he touched upon most of the plant families, classifying the grains according to their characters, though not in the form of a key. For example, he stated that the exine may cover the intine as a uniform membrane (*Canna*, *Strelitizia*, *Musa*, *Persea*), but it more often possesses thin spots. These may be closed by lids (Cucurbita). In this way he showed the phyletic distribution of all the known pollen characters, but perhaps the most valuable part of his paper is his excellent original drawings which accompany these descriptions.

Most of the work up to this time had dealt with pollen in general, taking for comparative studies pollen of widely separated species, generally so widely separated that little hint was offered of the transitions of form which are now known to exist between the pollen of different families. The result was that much of the evidence pointed to an absence of any parallelism between pollen forms and phylogeny. This was quite contrary to what should have been expected, particularly in the light of a growing feeling for the evolutionary origin of different biological forms, which was so greatly stimulated at this time by the publication of Darwin's "Origin of Species."

In order to find out to what extent the pollen forms agreed among the species of a single family, Sergius Rosanoff (1866) undertook to study the pollen of the Mimosaceae. One of the outstanding characters of this group is that the grains have a tendency to become united in various numbers. Therefore, while it is an excellent field for learning the effects of contacts of grains with each other, until these have been learned it is not a happy choice for discovering the relation of phylogeny to pollen form. Rosanoff found that among the Mimosaceae the mature grains may be single or firmly united in groups of 4, 8, 16, or higher numbers of cells and that the single grains bear no resemblance to the components of the compound grains. From this he came to the conclusion that, as Kölreuter had already stated, the similarity of pollen grains of different species does not always agree with their closeness in relationship. The weakness in this argument, of course, lies in the fact that the effect of the contact relations of the cells with each other in these compound grains almost completely suppresses the expression of their innate characters, just as in the case of tissue cells. Wood cells are wood cells no matter what plant they come from and differ far more widely from bast or parenchyma cells in the same stem than they do from wood cells of other plants, however distantly related.

Rosanoff's paper, apart from this conclusion, contains much of value. He found that the Mimosaceae, like the orchids which also have compound grains, could be classed into three groups: Group 1, those with single-celled grains, including *Desmanthus*, *Prosopis*, two species of *Mimosa*, and two of *Acacia;* Group 2, with grains in octads, including *Schrankia* and three species of *Mimosa;* and Group 3, those with 8, 12, 16, and 32 cells, represented only by *Acacia*.

The breaking up of the genus *Acacia* among the three groups is partly explained by the fact that the two species of *Acacia* (*viz.*, *A. leucocephala* and *A. latisiliqua*) having simple grains are not true acacias, the former being now referred to the genus *Leucaena*, and the latter to *Lysiloma*. It thus appears that all true species of *Acacia* have compound grains ranging from 4- to 16-celled and occasionally higher but generally in multiples of four.

Though he was able to discover no resemblance between the compound and the single grains, he did find that the single-grained forms have many similarities. All have a granular exine with the granules arranged in lines converging toward the poles; all have three longitudinal furrows equally spaced and converging toward the poles; and under the furrows are found conspicuous lumps of highly refractive material. In these characters they agree, which is strong argument in favor of the phylogenetic value of such characters.

Up to this time there had been minor disagreements among investigators as to the true nature of the openings in the exine or *Austrittsstellen*, as they had now come to be called with a full realization of their normal function as points of emergence for the pollen tubes. Von Mohl had regarded them as not true openings, citing the lids of the same material as the exine covering them in squash and passionflower pollen; Fritzsche had regarded them as true openings but closed from within by a *Zwischenkörper;* and Meyen had hypothesized the presence of a third layer which emerged under pressure from within to form the pollen tube. Though these differences of opinion were really not significant and were more a matter of the material under consideration than of any basic morphological character, Aloys Pollender (1867) undertook to settle the dispute.

He examined the early stages of developing pollen grains of *Cucurbita* and of some Onagraceae. In the former he observed that the positions of the future pores with their lids seemed to be clearly marked off on the exine, but when he put the grains in weak chromic acid, shrinking the contents away from the wall, the incipient pores disappeared (his figures are so drawn). From this he came to the conclusion that the appearance of pores on the grain had been caused by the flattened cells which he thought he saw underlying the exine and that it was their function to form the lid and eventually to push it off, permitting the egress of the pollen tube. He therefore named them lid cells, stating that Fritzsche had mistaken such for *Zwischenkörper*. In the Onagraceae he said that the bulging germinal papillae were the same cells under a slightly different guise.

Whether or not Pollender was fully aware of the revolutionary import of such a supposition, making of the pollen grain a pluricellular structure, is not clear, but it seems that such matters were of little concern to him. His chief interest seems to have been to show that von Mohl, Fritzsche, and Meyen had erred. His statements, however, did not stand long unchallenged, for Christian Luersen (1869) immediately grasped the import of

Pollender's statements and answered him in his "Zur Controverse über die Einzelligkeit oder Mehrzelligkeit des Pollens der Onagrarieen, Cucurbitaceen und Corylaceen." He made a lengthy and detailed study of the pollen of these groups, for the most part confirming the work of his predecessors and adding thereto. But of Pollender he was able to say without fear of contradiction that his conclusions were unjustified and entirely without foundation. He found that neither in pollen grains of the Onagraceae nor *Corylus* are separate cells present, nor are such cells found under the lids, as Pollender had pretended to discover. He concludes that, in the onagrads, Pollender probably mistook for a cell the space formed beneath the germ pore by the shrinkage of the contents, and in *Cucurbita* and *Corylus*, the thickening of the intine or *Zwischenkörper* underlying the pores. But perhaps more important was Luersen's discovery incidental to these studies that the pollen grains of *Epilobium* are frequently found in the anther firmly united in tetrads as they were formed in their mother-cells and corresponding in arrangement to the angles of a tetrahedron, always with the bulging germ pores of neighboring grains touching each other, in six pairs in the position of the faces of the tetrahedron. The importance of this lies in the fact that it shows that the three germ pores of such grains originate at the points of contact made by each grain with its three neighbors of the tetrad. This point is beautifully illustrated with original drawings. If Pollender's paper has any value, it lies in the retort that it called from Luersen.

In England at this time attempts were made by some investigators to comprehend the significance of the pollen forms which were being revealed by the recently improved microscopes, but these were for the most part frustrated by a total disregard on the part of these investigators for the work that had already been done on the Continent. Only one of these will be mentioned, for the others played no part whatever in the development of our science. M. P. Edgeworth (1877) published from London a little book entitled "Pollen." It would not be mentioned here at all, except that, strangely, it is today the most quoted of all the earlier works. The book purports to be a compendium of nearly all known pollen forms. Each species is described in cryptic form and illustrated. The descriptions are for the most part erroneous, and the figures are purely imaginary, having almost no basis in fact. Yet these bizarre and grotesque figures resembling nothing that could possibly exist in nature have been copied and recopied, published in journals, textbooks, and even in newspapers, to the great detriment of these vehicles. The book itself was republished in 1879.

It was well known at this time that an almost universal character of pollen grains was their great aptitude for absorbing water with a surprisingly large increase in their volume, but the mechanisms whereby such volume changes were accommodated had been entirely overlooked since the time when Kölreuter had stated that in certain types of grains the intine could bulge through round pores in the exine, so preventing the rupture of the latter as expansion took place. It remained, however, for the French investigator J. Vesque (1883), who thought of pollen grains as living dynamic entities and not just inert vehicles of fertilization, to explain the mechanical organizations of pollen grains for accommodating their ever changing volumes. He tells us that when a pollen grain loses water it shrinks. In the simplest cases it just contracts with the formation of surface cavities. This is what happens with the grains of grasses, for example, causing them to assume irregularly shaped pyramidal or conical shapes when dry. But in other kinds of pollen the structure of the grain is such that it retains its symmetry no matter how great the loss of water that it may undergo. This result is obtained by two different means: (1) by meridionally arranged, spindle-shaped areas where the membrane is thin and weak (He does not call these areas germinal furrows, nor does he say that they have anything to do with germination. That function—the only one which they were supposed, by all other investigators, to possess—did not even interest him. He says that when moist such a grain is spherical and the spindle-shaped areas are broad but that as the grain dries it becomes ellipsoidal and the thin-walled areas become narrower until finally their cutinized edges touch and transpiration is thereby greatly hindered. If a further loss of water continues, it makes the grain thinner and longer, at the same time inducing in its interior a proportionate negative tension which must eventually check the loss of water.); (2) by one or both polar surfaces, at first convex, tending to become flat or concave as water is lost, returning to their original convexity when a permiable part of the grain happens to come in contact with a moist surface. An example of this type of grain is beautifully illustrated by the grain of *Juglans*, in which one polar hemisphere bears the pores through which water may be transpired or absorbed, while the other polar hemisphere is covered only by a thin, flexible membrane which may be depressed or raised in compensation for loss or gain in volume.

Further on in the same paper Vesque makes the surprising statement that the arrangement of the spines and of the laminae and the networks, which decorate the surface of pollen grains, does not appear to depend upon the mode of development: "They seem to obey only a geometrical law which may be none other than the law of phyllotaxy extended to other projecting organs of the plant and to the law of economy for the net." Quite clearly he recognized the mathematical arrangements of the surface sculpturing and their underlying law of least surface configuration which is regarded as a very modern idea. "Nothing is easier," he says, "than to explain the complicated form of the grains of the Cichorieae in this way. These grains are covered with ridges perpendicular to the surface, which form a regular network of pentagonal and hexagonal spaces." He did not quite grasp the controlling mathematical proportion between the number of pentagons and of hexagons which may be placed on a spherical surface but thought that this relation had something to do with the deviation of the shape of the grain from the truly spherical form, stating that "the number of hexagonal spaces will be greater as the grain approaches the cylindrical form"—offering this as the explanation for the fact that, of the 21 faces on the grains of *Lactuca*, 9 are hexagonal, whereas, in reality, the proportion between the hexagonal and pentagonal faces is in this case one of the few choices which are mathematically possible on a sphere when the total number of faces is 21. Nevertheless, Vesque here introduced for the first time into the study of pollen grains the mechanistic conception that their underlying forms are controlled by purely physical and mathematical laws.

In the meantime, the subject of the development of pollen grains was being vigorously prosecuted by Eduard Strasburger. Following a number of short papers on the subject, he published a masterly sketch in his "Ueber den Bau und das Wachsthum der

Zellhäute" (1882). He chose for study representative forms as dissimilar as possible, so as to exhaust—as he says—the multiplicity of different combinations. The pollen of *Malva* was studied in the minutest detail, from the anlage of the mother-cells up to the completed form and even to its germination and tube formation. Using this as a background, he made comparative studies of *Geranium*, *Guarea*, *Oenothera*, *Clarkia*, *Epilobium*, *Pinus*, *Larix*, and many others. This work he extended and greatly improved in "Ueber das Wachsthum vegetabilischer Zellhäute" (1889). These two works cleared up almost completely the perplexing subject of pollen development, and they form the basis of our knowledge of it today. But dealing mainly with development they are somewhat aside from our central theme so cannot be dwelt upon further at this time. In passing, however, it should be noticed that Strasburger here introduced a curious conception of the exine: "I call *exinium* the first coat entirely covering the whole spores and pollen grains, *intinium*, a second inner coat which is separated from the first." This was not according to the conception which we have seen that Fritzsche had when he coined the words exine and intine, and it led to some confusion later on.

We must also pass by, with little more than a word of mention, two very interesting papers by Louis Mangin (1886, 1889). The first of these deals with the physiology and chemistry of the germination and growth of pollen; the second, with its structure and development, and seems to have been inspired by the work of Strasburger. Mangin states that the intine in most cases consists of cellulose and pectose. These two substances may be mixed in various proportions, but more often the cellulose is concentrated in the inner layer, and the pectose in the outer layer of the intine, the two merging in between. Beneath the *Austrittsstellen*, where the intine is generally greatly thickened, it consists principally of pectose, such thickenings being the *Zwischenkörper* of Fritzsche. In this same paper Mangin takes Strasburger to task for his statement that in the grains of *Allium fistulosum* the intine is not formed. Instead, says Strasburger, the exine is continuous throughout and cutinized except in the region of the single germinal furrow, where it is greatly thickened but not cutinized. This part which Strasburger regards as a thickened, noncutinized part of the exine, he states, gives rise to the pollen tube. Mangin stoutly contradicts this, stating that the intine is continuous and gives rise to the pollen tube, while the exine is a discontinuous layer, not covering the region of the germinal furrow. These differences of opinion caused a lengthy controversy between the two investigators. Strasburger's viewpoint, while decidedly heterodox, conformed to his conceptions of intine and exine which he had clearly defined some years previously, while Mangin's viewpoint conforms to the conception that was generally accepted and was clearly that intended by Fritzsche in his original definitions of the words.

While the works of Strasburger and Mangin were not in themselves strictly morphological, the light that they threw on pollen structures made possible further advances in pollen morphology. Most attempts at working out anything like a comparative morphology had hitherto failed, because the structures of pollen grains were not fully understood and had not been clearly defined. But the work of Strasburger and Mangin had so cleared the field that it now awaited a really comprehensive comparative morphological study. This hiatus was soon filled by Fischer in 1890.

Fischer.—This scientist Carl Albert Hugo Fischer was born in Breslau in 1865, the second son of Robert Fischer. His earliest education was received at a private school in his native city. From there he went, in 1875, to Michaeli where he attended the gymnasium of Maria Magdelena until 1883, when he reached his majority. Afterward he attended the University of Halle, coming under the tutelage of such men as Grenacher, Kirchhoff, and Kraus. In 1886 he returned to Breslau, and under the direction principally of F. Cohn but also of Engler and Heidenhain he took his degree of Doctor of Philosophy in 1889 with his thesis, "Beiträge zur vergleichenden Morphologie der Pollenkörner." After the publication of this work it appears that Fischer paid no further attention to pollen, his interests centering mainly around colloidal chemistry, plant nutrition, soil chemistry, and applied botany. In 1898 he published an article on inulin and, in 1927, "Bau und Eigenschaften pflanzlicher Kolloïde," both in Cohn's "Beiträge für Biologie der Pflanzen." In 1930 he published "Landwirtschaftliche Bacterienkunde." He is at present living in Berlin and actively engaged in the study of plant physiology, floristics, and allied subjects.

In his "Beiträge" Fischer states that, finding the field of comparative morphology of pollen by no means exhausted, he set himself the two problems: (1) "How is the outer layer of a pollen grain formed?" and (2) "In what way do plants which are related in their outward form agree in their pollen-grain structures?"

FIG. 9.—Hugo Fischer, 1926.

But while he was busy with these subjects there came into his hands the second edition of Edgeworth's book (1879) and Strasburger's "Ueber das Wachsthum vegetabilischer Zellhäute" (1889). The second had so completely exhausted the subject of the development of pollen grains and in such a fashion that Fischer felt that no further study of that subject was necessary. Of Edgeworth's book, however, he says that it is quite worthless because the author makes so many mistakes that none of his work can be accepted without proof.

Fischer studied his grains both wet and dry in order to learn the effect of expansion on their shape, and many of the grains he embedded in gum arabic and sectioned with a razor staining with appropriate aniline dyes. In this thorough fashion he studied over 2,000 species of pollen in 158 families—a much more complete study than any which had hitherto been made.

He was impressed at the outset by the fact that pollen grains are always single cells. People sometimes speak of pluricellular or compound grains, he says, when what is really meant is "cells grown together." Like other cells, pollen grains have three principal parts—nucleus, plasma, and wall—and the characters whereby the different kinds of pollen are distinguished are cell characters, the properties of the wall or, more properly, of the exine.

The exine, he says, generally reveals the possession of a cuticle which, in its chemical reactions, shows much similarity to protein substances, staining an intense brown with iodine and taking aniline dyes in the same ways that proteins do. This substance also gives the xanthoproteic and Millon's reactions which characterize proteins but differs from most proteins in being insoluble in alkali. In this latter character, however, the cuticle differs from suberin and cutin, which it otherwise resembles very closely. Fischer states that the exine is insoluble in concentrated nitric, hydrochloric, or sulphuric acid. It also resists gastric digestion, for upon examining the excreta of chafers which had been feeding on pollen, he found grains with their exines intact though their nutrient contents and intines had been dissolved away. Almost the only chemicals that he could find which would dissolve the exine were eau de Javelle and chromic acid; and to these reagents the different species of pollen showed marked differences in their reactions; with some the exine dissolved easily, but with others, *e.g.*, *Pinus*, it could be dissolved only upon prolonged boiling. In concentrated sulphuric acid the exine turns red, and this, Fischer says, is due to the presence of sugar, as had already been pointed out by Strasburger (1887) in the case of proteins. Structurally, Fischer says, Fritzsche had already pointed out that the exine usually consists of two layers. The inner is more highly refractive to light and has a weaker affinity for aniline dyes than the outer which carries most of the sculpturing responsible for the diversity of pollen forms.

Fischer's classification of these forms is as follows:

I	*A.* Exine absent
	B. Exine present
II	*a.* Uniform throughout
	b. With *Austrittsstellen*
III	1. *Austrittsstellen* round
IV	2. *Austrittsstellen* as furrows
	c. With germ pores
V	1. in the furrows
VI	2. free on the surface
VII	With one or more lids.

Fischer uses the word *Austrittsstellen* in a more restricted sense than did his predecessors, applying it only to thin spots in the surface as distinct from actual holes through the exine, the germ pores.

In his classification he makes no use of the compounding of grains, as previous investigators had done, for, he says, it has no significance—some species may have compound grains, while their nearest relatives may have single grains, *e.g.*, species of *Typha* and *Jussieua*. But a most striking observation that he made in his study of compound grains was that in symmetrical tetrads grains with bipolar axes are so arranged in relation to one another that the axis of each grain is directed toward the middle of the group, (*e.g.*, Ericaceae, *Drosera*, and *Jussieua*). When each grain has only one *Austrittsstelle* it faces outward from the other grains (*e.g.*, *Philydrum*, *Victoria*, and *Drimys*). We may perhaps call this Fischer's law, for I believe that no one before had observed this important relation between the forms of grains and their spatial relations in their tetrads, though Fritzsche had come very close to doing so. It is to this law that pollen grains owe most of their basic form characters.

The body of Fischer's work consists of a critical examination of the pollen of about 2,200 species arranged according to his classification given above. And from these studies he was able to draw the following conclusions:

Pollen grains of related species are generally similar; indeed, a single form of grain often runs through the whole family, and the species of a genus can hardly ever be distinguished from

each other by their form, though they may occasionally be by their color.

Some families have more than a single basic form. Sometimes unrelated plants have similar pollen.

In the general evolution of pollen grains there has been a progressive strengthening of the exine and, at the same time, the formation of prearranged places of exit for the pollen tube, but, in both of these, sometimes a reversion has occurred. The strengthening of the exine takes place not merely through the accumulation of thicker material but more especially through the addition to the ground membrane of spines and reticulations. Thus the grains of the gymnosperms and monocotyledons generally have an exine of simple structure, while those of the dicotyledons generally have an exine which is more complicated, culminating in the spine-covered grains of the Compositae and the reticulate grains of the Cichorieae.

The pollen grains of the gymnosperms, monocotyledons, and lower dicotyledons are generally monomerous, while those of the higher dicotyledons are generally trimerous. This condition results from their position in the tetrad, which is generally in one plane in the former and tetrahedral in the latter. But there are many exceptions, so this criterion may be employed only with caution in classification.

Adaptation to insect pollination is accomplished by the presence of oil, which Fischer believes is secreted through the exine, and by the formation of spines. But the pollen of those plants which have reverted to anemophily differs from that of their related entomophilous species in having grains with thinner and smoother exine but generally standing in other respects close to the pollen of their relatives, as, for example, the anemophilous pollen of *Thalictrum*, *Artemisia*, and *Ambrosia*, though they may be entirely different, for example, the smooth, furrowless grains of *Populus* as compared with the reticulate, three-furrowed grains of *Salix*.

These few very concise statements of Fischer's summarize briefly and adequately the phylogenetic value of pollen-grain characters. They were first noticed by Grew and Malpighi. One by one they were laboriously brought to light and sifted out by subsequent investigators. There were many apparent contradictions, and many mistakes were made. But through the brilliant researches of such men as Mirbel, Purkinje, von Mohl, and Fritzsche, to mention only a few of the leading names, our understanding of pollen forms gradually took shape; it remained, however, for Hugo Fischer, taking nothing for granted, going always back to nature, to round out our knowledge and give us the morphology of pollen grains in its modern form.

CHAPTER II

METHODS OF COLLECTING POLLEN IN LARGE AMOUNTS

When it is necessary to secure pollen in large amounts, as for the preparation of hayfever-pollen extracts or various other chemical work, special methods must be employed according to the nature of the plants from which the pollen is to be obtained.

In general, it may be said that *it is always best to collect from the first flowers to open* rather than from those that bloom toward the end of the flowering season, because the late flowers are usually less vigorous and are likely to be infested with insects. With flowers which are particularly susceptible to pollen-eating insects, *e.g.*, the oxeye daisy and sorrel dock, the first flowers may be nearly free from such insects, but as the flowering season advances they become more and more infested with them, finally reaching a stage where there are so many insects that practically all of the pollen is devoured as fast as it is released from the anthers. With most forest trees it is essential that the flowers be gathered as soon as the first of them open, because the flowering period is often so short and so much under the influence of local weather conditions that if the weather turns suddenly warm and dry, anthesis may be completed within two or three days. Thus it is with early flowering trees, such as the pines, junipers, elms, and poplars; unless advantage is taken of the first opportunity to collect their flowers, the opportunity is likely to be lost entirely for the season. With the early flowering trees the factors that finally bring about anthesis are generally temperature and humidity. The flowers reach the bursting point and remain in this condition until the arrival of a warm, dry day when they suddenly open and complete their pollination within one or a very few days. Advantage should be taken of this fact to collect the flowers of such plants in the cool, damp days immediately preceding anthesis.

The method employed in handling the flowers depends to a large extent upon whether they are wind- or insect-pollinated and whether they will continue to live and shed their pollen after cutting or whether, as sometimes happens, pollen shedding ceases as soon as the flowers are cut.

Natural Shedding.—Nearly all anemophilous, and a few entomophilous, plants can be made to shed their pollen quite naturally indoors. Such, for example, are the conifers and most other gymnosperms, the Gramineae, Cyperaceae, Typhaceae, Chenopodiaceae, Amaranthaceae, most of the early flowering trees, the Ambrosiaceae, and *Artemisia* and, among the entomophilous plants, some species of goldenrod, thistle, and sunflower. In dealing with plants of this class the flowering stems are cut off and placed in water in the shedding room. For this purpose it is convenient to use long, shallow pans about one foot wide in which a central bar has been arranged lengthwise to act as a catch to hold the cut ends of the stems beneath the water. The plants are placed in these pans in a nearly horizontal position so that the flowering tips lean out far enough to be clear of the edges of the pans. Sheets of paper are then placed under the flowers to catch the pollen. The flowers should be gently tapped once a day until the pollen ceases to drop off; then the papers should be drawn out, and the pollen removed.

Most of the grasses can be handled in this manner. They should be cut in the early morning—if possible while the dew is still on them. The leaves should be stripped off in the field, and the stems trimmed to the proper length to fit the shedding pans. They should then be bundled loosely together, and their stems kept moist by the addition of water or, during very dry weather, by keeping them immersed in buckets of water until the shedding room is reached. The cut plants should not be stacked together for a longer time than necessary, and on no account may they be bundled tightly, because respiration is rapid during this period of their growth, and so much heat and moisture are generated that the plants may be "burned" and completely destroyed in less than an hour.

Collecting should be done in the morning. Many grasses exhibit a marked periodicity in their shedding. Some, for example, orchard grass, shed most abundantly early each morning, beginning as soon as the warmth of the sun's rays is felt, falling

off toward noon, and ceasing in the afternoon. Others begin late in the day; for example, redtop begins to pollinate rather suddenly at about three o'clock in the afternoon and continues for only an hour or two. In either case the plants should be collected in the morning and placed in the shedding pans as quickly as possible, because the drying that sets in as soon as they are cut accelerates the opening of the anthers and causes the bulk of the day's pollen to be shed soon after.

Some grasses, of which the Indian rice and tall oat grass are examples, will shed little or no pollen after being cut. Nevertheless, these should be placed in the shedding pans as described above. After two or three days most of the anthers will be found to have dropped off unopened, and the rest can be removed by gently shaking the inflorescences. These anthers should be brushed up, partly dried, and shaken on sieves to remove any pollen that might happen to be freed from them. In order to obtain the pollen still remaining in the anthers they should be passed through a coffee mill with the plates adjusted just tight enough to roll the anthers and loosen the pollen. If this is done properly and the anthers are not too dry and brittle, most of the pollen will be set free and may be separated from the crushed anthers by sifting.

Pollen obtained by this method always contains a large proportion of immature grains, which are said to be anaphylactogenically less active than those that are mature, and some fragments of anthers which are totally inactive. The proportion of these will depend upon the skill and care with which the grinding and sifting are done.

Plant-breeder's Method.—Grasses and other plants which fail to shed naturally indoors can sometimes be handled by a plant-breeder's method. This consists of tying paper bags over the inflorescences in the field, just as they are starting to pollinate. Special bags may be obtained for the purpose which are proof against destruction by moisture but porous enough to permit the escape of the moisture which otherwise tends to accumulate within. The bags can be left on for only a few hours, after which they must be removed, and the pollen emptied out and dried.

Most insect-pollinated flowers offer more difficulty than those that are wind-pollinated, except the very few kinds that will shed naturally from pans of water. For roses we may employ the following special method. The flower buds are gathered just as they are about to open. They are then dried slowly on sheets of paper in a warm, dry, and well-ventilated room. The corollas, which still remain tightly closed, are pulled off from their receptacles, and the pollen emptied out on to a sieve and brushed through to separate it from the anthers and other floral parts which come with it.

Carbon Tetrachloride Method.—Most entomophilous plants, in which the pollen is sticky and remains enclosed within the flowers, will yield to the carbon tetrachloride method (Wodehouse, 1916), which is briefly as follows: The staminate flowers, after being dried but not completely desiccated, are removed from their floral receptacle and freed as far as possible from all extraneous material. The mass of flowers is then soaked in carbon tetrachloride in an open pan or mortar and gently beaten with a pestle, to loosen the pollen from the anthers or pollen tubes; the whole is then emptied on to a piece of fine muslin stretched across another pan, and the carbon tetrachloride pressed out. The liquid carries the pollen through the muslin, leaving the crushed flowers behind. These should then be washed repeatedly until all the pollen is loosened and suspended in the carbon tetrachloride. The suspension is then poured on to a Büchner funnel to which a piece of smooth filter paper has been fitted, and the carbon tetrachloride drawn rapidly through by suction, leaving the pollen collected on the filter paper in a solid cake. This should be washed with fresh carbon tetrachloride, to free it from lipoid substances, and dried by sucking air through it. The cake is then broken up, and the pollen sifted through a 200-mesh sieve and completely desiccated. Pollen prepared by this method is generally contaminated to some extent with pollen of foreign species and plant fragments, but under favorable conditions these occur in only negligible quantities.

Desiccation.—Complete and prolonged desiccation must never be omitted, by whatever method the pollen is obtained. Pollen collected on the papers in the shedding rooms may appear to be quite dry when brushed up but, if confined in glass jars without a preliminary complete desiccation, after a few days it becomes caked and useless. Furthermore, there is evidence to show that even air-dried pollen deteriorates slowly, but this may be prevented by completely desiccating and enclosing it in airtight containers.

Therefore, as soon as possible the pollen should be brushed up, sifted, and dried in a vacuum desiccator over calcium chloride or sulphuric acid until no more moisture can be removed, when it should be transferred to airtight containers with as little exposure to the air as possible. Even after desiccation pollen may take up moisture if exposed to the air. Indeed, the absorption of moisture from the air is so rapid with some kinds of pollen that it is difficult to weigh them in ordinary atmosphere.

CHAPTER III

PREPARATION OF POLLEN FOR MICROSCOPIC EXAMINATION

Methyl-green Glycerin Jelly.—A small amount of pollen, about as much as can be picked up on the flat end of a toothpick, or less, is placed on the center of a microscope slide, and a drop of alcohol added and allowed partly to evaporate. A second and third or even fourth drop may be added if necessary. The alcohol spreads out as it evaporates and leaves the oily and resinous substances of the pollen deposited in a ring around the specimen. The oily ring is wiped off with cotton moistened with alcohol, and, before the specimen has had time to dry completely, a small drop of hot, melted methyl-green glycerin jelly is added, and the pollen stirred in with a needle and evenly distributed. During the process the jelly is kept hot by passing the slide over a small flame, heating it just enough to sting but not burn the knuckle, which may be used to test its temperature. A No. 0 cover glass, which has been passed several times through the flame while held vertically with the forceps, is then placed over the specimen, and the slide gently heated. If the amount of jelly has been judged correctly, the cover will settle into position, with the gelatin reaching its periphery just when the pressure of the cover begins to be taken up by the pollen grains. This amount must be learned by experience and accurately gauged, because a smaller amount leaves the grains crushed or flattened by the cover or the mount incompletely filled, and a larger amount causes the preparation to be too thick for use with oil-immersion lenses.

If naturally shed pollen is not available, satisfactory material can generally be obtained from herbarium specimens, provided they were quickly and completely dried. Often it is only necessary to tap the dry flowers over the slide or crush a few anthers on it. If pollen cannot be removed in this way, a few anthers or, with the Compositae, a few florets may be removed from the

specimen and placed on the slide. These are then moistened with alcohol, followed by a drop of water, and heated to boiling. The pollen may then be teased out, and the anthers and other debris removed, leaving the pollen in the water. The water is then drawn off with cotton or filter paper, and the jelly added as before.

The glycerin jelly is prepared according to the method of Brandt* which is as follows: Soak some gelatin for 2 or 3 hr. in cold water, pour off the superfluous water, and heat until melted. To 1 part of this add 1½ parts of glycerin and, while still hot, filter through spun glass pressed into the lower part of a heated funnel. Add 2 or 3 per cent phenol. Still keeping the mixture hot and fluid, add drop by drop a saturated solution of methyl green in 50 per cent alcohol, until the glycerin jelly becomes fully as dark as green ink.

Methyl-green glycerin jelly may also be made by adding the dye, as indicated above, to a good commercial preparation of glycerin jelly, of which there are a number on the market.

The relative proportions of glycerin and water are so balanced in glycerin jelly that the majority of pollen grains when placed in it are fully but rarely over expanded, and they never burst and extrude their contents, as is usually the case with ordinary aqueous mediums.

Contrast Stain.—When treated as above, the methyl green stains only the exine, leaving the intine and cell contents uncolored. If it is desired to show a contrast between the exine and cell contents, the pollen may be given a preliminary staining on the slide with weak aqueous eosine. The contrast thus obtained is striking and brilliant and therefore excellent for demonstration purposes but of no other advantage.

Pollen grains mounted in glycerin jelly and stained with methyl green have a peculiar habit of developing greater brilliancy during the first few days after they are made, probably through a slow, selective adsorption of the dye from the embedding medium. It is therefore advantageous to make up the slides a day or two before they are required. They retain their brilliancy for several months unimpaired but unfortunately are not permanent, for the dye fades slowly out after a period varying from about 9 months to 2 years, after which they are

* Lee, "Microtomist's Vade Mecum, 7th ed., p. 242.

entirely bleached. Such preparations may always be restored, however. To do this the slide is heated, the cover glass removed, a small drop of hot methyl-green glycerin jelly is worked in with a needle, and the preparation covered with a fresh cover glass. Specimens so rejuvenated are just as good as new, but the labor of recovering them is perhaps a little more than would be required to make them anew from fresh pollen. Nevertheless, the method is valuable in cases where the pollen is no longer available.

Aqueous fuchsin has the same selective properties as methyl green, is much more vigorous in its action, and is *permanent.* The only objection to its use is that the red color is theoretically not quite so satisfactory for observation with high-power lenses as the blue color, though in actual practice there is little difference. It may be used in place of methyl green as above, or according to the method of Fischer (1890) as follows: After the pollen has been washed with alcohol on the slide, a drop of weak solution of basic fuchsin in water is added, and the pollen stirred in with a needle. As soon as the grains have taken up all the dye, the water is drawn off with cotton, and, without letting the preparation dry, a drop of melted glycerin jelly is added, and the pollen stirred in, keeping the slide warm. It is then covered with a No. 0 cover glass.*

Atmospheric Pollen Slides.—Methyl-green or fuchsin glycerin jelly serves very well for catching atmospheric pollen and making pollen counts and identifications. A small drop of the melted glycerin jelly with the dye added is placed on a slide, spread out to occupy an area about equal to, and of the same shape as, the cover glass which is to be used in finishing the mount. The slide is exposed in a horizontal position protected from rain and sun by a shelter raised at least 4 in. above it. After 24 or 48 hr. the slide is brought into the laboratory and examined with a hand lens, and any extraneous material, like soot or sand, which, if present, might prevent the cover glass from fitting into place, is removed. The slide is then heated, the temperature being controlled with the knuckle as before, until any excess moisture which may have accumulated during exposure is driven off. It is then covered with a No. 0 cover glass.

* A very satisfactory glycerin jelly for this purpose is that prepared by Eimer & Amend, New York.

In order to make the pollen count from such a slide, it is placed on a microscope provided with a mechanical stage and passed from left to right and from right to left, progressing the width of a single field in a forward direction with each passage from side to side. For this purpose it is most economical of time and labor to use a low magnification (about 150 diameters), and whenever a pollen grain is found that cannot be identified at that magnification, a higher objective is swung in, the identification made, the high objective then replaced by the low, and the counting continued until the entire surface has been covered.

Examination of Dry Pollen.—When it is desired to discover the unexpanded shapes of pollen grains they may be observed dry in air with or without a cover glass. Such observations are also useful in seeing how much oil naturally occurs on the surface of the grains, also as a check against the method to be described below. With dry pollen in air, magnifications much over 200 diameters can scarcely be used to advantage; nevertheless, even at this magnification the shapes of the grains can be learned and something of the mechanical action of their organs of volume-change accommodation. If, however, a detailed examination of the grains in their unexpanded condition is desired, they must be stained and brought into a medium of suitable refractive index. This may be done as follows:

Aniline-oil Gentian-violet Method.—Place the pollen on the slide as before and add two or three drops of aniline oil which has been tinted with gentian violet only to a pale-purple color. Heat gently over a small flame, controlling the temperature with the knuckle as before until the grains become deeply stained; allow the slide to cool to room temperature; draw off the excess oil with filter paper; wash by repeatedly adding xylol and drawing it off until all the oil and unabsorbed dye have been removed; then add a drop of Canada balsam and cover. This method presents the grains unexpanded but brilliantly stained and in a medium eminently suited for observation with high-power oil-immersion lenses.

CHAPTER IV

POLLEN STATISTICS

A BOTANICAL AND GEOLOGICAL RESEARCH METHOD

BY GUNNAR ERDTMAN, *Stockholm**

Pollen statistics is the method of tracing the history of forests by a study of the occurrence of fossil pollen grains in peats and sediments. The method was elaborated some 20 years ago by the late Prof. G. Lagerheim of the Department of Botany at the University of Stockholm and by Dr. L. von Post, now professor of geology at the same university.

Pollen statistical methods include work in both the field and the laboratory. The field work is done chiefly during the summer. During the winter, however, when the lakes are frozen over, samples of bottom sediments can be obtained from them by means of borings through the ice. Several types of auger are available; but of these the one that is coming into most general use is the Hiller peat auger, manufactured by Beus and Mattson Company, Mora, Sweden (Fig. 10). This company offers the auger in two different models—a smaller one, with the chamber in which the peat sample is collected 32 cm. long and extension rods 100 cm. each; and a larger model, with the chamber 40 cm. long and extension rods 150 cm. each. If the field work is to be carried out with the help of an assistant, preference should be given to the heavier auger, which is more reliable than the lighter one. The field apparatus also includes a spade, big knife, nickel-plated forceps with smooth ends, glass tubes in which to keep the peat samples (about 7.5 cm. long, 1.3 cm. inside diameter, and corked at both ends), diopter compass, and a geodetical set for taking levels and distances.

* The essential parts of this chapter were included in lectures given at the University of Chicago and the University of Michigan, July, 1931, and in a note in *Science*, March, 1931. The section on technique is an abridgment of an article by G. Erdtman and H. Erdtman (1933).

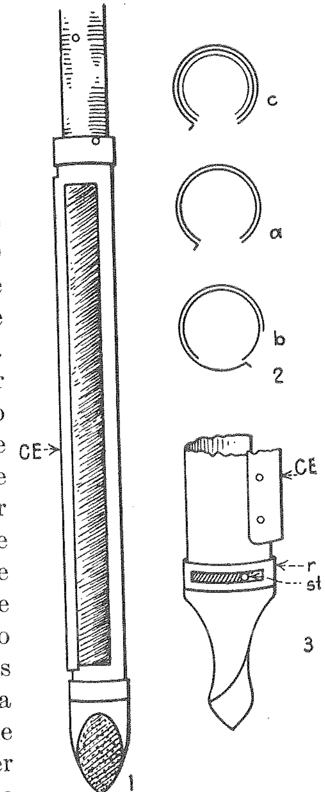

FIG. 10.—Peat auger, Hiller model. 1. Lower end of auger with chamber open. The cutting edge, *CE*, should be fairly sharp. The opposite edge may be marked at intervals of 5 cm. 2. Cross section of the chamber: *a*, open, *b*, closed, *c*, to show the position of the zinc lining which is represented in the sketch by the inner arc. 3. Lower part of the chamber and screw point. The outer sleeve of the chamber is riveted to the ring, *r*, but the inner is free to revolve within. *st* is a pin fastened to the inner sleeve; it can traverse the length of the slot in the ring, thus checking at the proper places the opening and closing movements of the sleeve. (*Adapted from Kräusel*, 1929.)

Peat samples should be taken from the cleaned walls of peat hags if such are available, but, if not, borings have to be made. To do this a big sod is removed from the surface of the bog with the spade. From the walls of the sod the first samples are taken, say, from 2, 5, 10, 15, and 20 cm. below the surface. Then the auger is put in the hole left by the sod and forced down into the peat. Meanwhile the handle of the auger should be kept turning slightly to the right (clockwise) to prevent the container from opening. When the desired depth is reached the container is opened, and a good compact core obtained, by turning the handle swiftly about four revolutions to the left. It is then closed again by two turns to the right, and the auger is pulled up out of the peat, giving it a slight continued revolution to the right to be sure that the chamber will stay closed until it reaches the surface. The core is then removed from the chamber, and the outer layer, which might have become contaminated with material from higher levels, is removed with the knife. Samples are then taken from the core with the forceps at regular intervals, *e.g.*, at every fifth centimeter.

It is sometimes advantageous, when working with an auger of the larger model, to place a thin, removable zinc lining inside the container (see Fig. 10). When a boring is made the lining gets filled. The lining with the core of material enclosed is then removed from the chamber of the auger and brought intact into the laboratory. By the proper use of zinc linings a complete series of such cores from the surface to the bottom of the bog can be obtained. Drying and shrinkage of the material are prevented by glycerin, and, from such cores, samples for pollen-statistical investigations may be picked out at any time and from any part of the cores. Sometimes one sample from every second foot of the series of cores is sufficient; at other times a dozen samples from one inch of core may be required. It is impossible in the field to be sure of the best intervals at which the samples should be taken; the use of the linings, however, solves the problem.

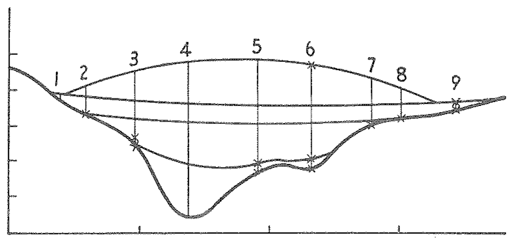

FIG. 11.—Cross section of a bog. For explanation see text.

It is convenient to have the spade standing with its blade thrust into the ground near the boring and to put the lower end of the auger with its chamber through the handle of the spade while the samples are being taken from the chamber. Figure 11 shows, schematically, a section of a bog the stratification of which has been made out from serial borings. A complete series of peat samples would most suitably be gathered from point 4, and additional samples for the study of the growth of the bog and the composition of the Sub-recent pollen flora, etc., from the places marked with crosses.

When preparations for microscopical work are to be made, a small amount of peat is taken from one of the samples, laid on a slide, and mixed with 10 per cent caustic potash. The slide is then held with a clothespin, and the mixture carefully boiled over a small alcohol flame until the greater part of the water has evaporated. Some drops of glycerin are then added and mixed with the peat, and a part of the mixture is removed to another slide and covered with a cover glass. The pollen grains are then

counted by the use of a microscope with a mechanical stage. A magnification of about two hundred times is required for counting, and a high-power lens for the study of the finer structural details of the grains. Some sediments need no boiling, and preparations are made by simply mixing a part of the substance with distilled water. Calcareous material is treated with dilute hydrochloric acid and minerogene earths, and even rather coarse sand can be subjected to pollen analysis if centrifuged and treated with hydrofluoric acid. Trustworthy percentages are obtained, as has been proved for Sweden, by counting about 150 pollen grains. As to samples from northern Canada and other countries with only a few tree species of which pollen is preserved in bogs, fairly reliable percentages can be obtained by counting 100 or even fewer pollen grains. It is, on the other hand, desirable that more than 150 pollen grains should be counted in samples from districts with a great number of tree species (*e.g.*, great parts of the United States).

The frequency of the pollen grains of hazel, willow, and other species, which are more or less confined to the undergrowth of the forests and are not regarded in these studies as forest trees, is calculated separately and expressed as a percentage of the sum of the pollen grains of the forest trees proper. Thus a willow pollen frequency of 138 per cent indicates that the number of willow pollen grains in that sample was greater than the sum of the pollen grains of the forest trees. The frequency of *Sphagnum* spores, tetrads of Ericaceae, etc., is expressed in the same way. It is useful, too, to have a record of the *pollen frequency* per square centimeter, *P F*, from each preparation.

The percentages, *i.e.*, the relative frequency numbers, that are found for the pollen species in a sample constitute the *pollen spectrum* of the sample. The pollen spectrum shows to a certain extent the proportions of the different kinds of pollen grains which settled on the bog when the layer, from which the analyzed peat was taken, formed at the surface.

On the basis of a series of pollen spectra from a boring in a bog a *pollen diagram* may be constructed, with the depths of the peat plotted as ordinates and the pollen percentages as abcissas corresponding to those levels where the samples were taken. In a pollen diagram the curves for the single species or for a group of species give both a visual representation of the composition of the pollen flora and the oscillations with regard to frequency, which have taken place reciprocally between the pollen curves during the formation of the bog.

When a sufficiently close network of pollen-diagram stations has been completed for a country, and when the diagrams, with the aid of archeological data from the bogs or in other ways, have been properly dated, then it is possible, with practically the same accuracy as in the case of modern forests, to reconstruct on maps the distribution of the forest trees and the changes of the forest types from one area to another and from one region to another during different periods.

AN IMPROVED TECHNIQUE OF POLLEN ANALYSIS

The process of digesting peat by means of boiling with dilute alkalies, which at present is in general use, suffers from several sources of error, both theoretical and practical. Indeed, the composition of the fossil pollen content can be shown to vary according to the method of preparation of the samples for investigation.

If peat is prepared for microscopic investigation by boiling with alkali—rather a severe treatment—a source of error is introduced. Sometimes the peat is boiled with dilute alkali until most of the water has evaporated (this treatment may be repeated); sometimes it is boiled less intensively. According to the difference of treatment the results are often not strictly comparable. Furthermore, the peat is frequently inadequately digested by the alkali, and some pollen will consequently escape observation because partly or wholly obscured by undigested debris. Small pollen grains escape attention more readily than larger ones, which therefore tend to be overrepresented. This source of error is well known* and may in certain cases cause considerable distortion of the pollen percentages.

Another source of error is connected with the final covering of the slide with the cover glass. It is easy to demonstrate that the relative frequencies of the pollen grains in an artificial mixture of pollen grains (or in a peat preparation) may change to some extent according to the force with which the cover glass is pressed against the slide. This distortion will occur if some of the

* Cf., *e.g.*, Assarsson and Granlund, pp. 80, 81, 1924.

material is squeezed out at the edges of the coverglass and seems to be due mainly to the varying sizes of the pollen grains.

Another drawback to the alkali method is, the difficulty of concentrating the pollen grains sufficiently for analyses of peat samples of low pollen content to be made reasonably quickly.

Among the constituents of peat are cellulose and hemicellulose, which on hydrolysis by acids are transformed into water-soluble products, *e.g.*, glucose. Other constituents are lignin and humic acids, both of which cannot be hydrolyzed by acids but are easily destroyed by oxidation. Combining the method of hydrolysis and oxidation we have been able to remove these constituents of peat and isolate the more resistant elements, *viz.*, pollen, spores, and fragments of cuticles. For the oxidative destruction of lignin and humic acids, treatment with chlorine dioxide* was found useful; the remainder of the peat was then treated with cold, strong sulphuric acid in order to hydrolyze the polysaccharides.

In our preliminary experiments we used a homogenous standard peat prepared from slightly moldered *Sphagnum* peat, the age of which is estimated to be about two thousand years. It was rather difficult to analyze by the alkali method, but when treated according to our acid-oxidation method the pollen grains and spores, etc., were obtained almost free from peaty debris.

The new method may perhaps at first seem to be rather complicated, since it involves the use of different chemicals and a centrifuge. In our opinion this is quite outweighed by its advantages, particularly the possibility of concentrating pollen grains and other microfossils to an extent far beyond reach of the alkali method. This advantage is particularly manifested in the study of any peats which for one reason or another have a low pollen content. In this connection some Canadian and other peats have already been mentioned, and others might be added, such as the Greenland peats which are situated beyond the distribution area of forest trees. With the use of the new method it has also been possible to establish the presence of pollen grains which are destroyed by the alkali treatment, or which, if not destroyed, it would have been very difficult and time wasting to have counted in a preparation made by the alkali method.

The Proposed New Method of Preparation of Peat for Pollen-statistical Purposes.—In order to obtain a homogeneous stock of

* Schmidt and Graumann, 1921.

peat for a comparative study of methods dealing with the isolation of the pollen grains in peat, the sample of slightly moldered *Sphagnum* peat was mixed with cold 10 per cent sodium hydroxide solution and stirred until a semiliquid mass was obtained. After a few hours it was pressed through a metal net (meshes, 4 mm.) in order to remove coarse debris such as twigs, etc. After acidification with dilute hydrochloric acid (1:1) the peat was filtered with suction on a Büchner funnel and washed with water until the filtrate gave only a weak test for chlorine ions with silver nitrate. The peat was then spread on glass plates and dried at a temperature slightly above room temperature. The dried peat was carefully ground in a mortar and sifted (meshes, 0.4 mm.).

The peat thus obtained was used as a standard peat in our experiments and was first subjected to pollen analysis by means of the alkali method (boiling with 10 per cent sodium hydroxide until most of the water had evaporated, washing with distilled water, and centrifuging to remove the dark substances dissolved by the sodium hydroxide). Analyses showed the material to be homogenous enough to be used as a standard peat for methodological experiments (Table I). The uniformity of the five pollen

TABLE I

Pollen	Analyses					Extreme percentages	Average percentages
	1	2	3	4	5		
Alnus	15	11.5	14	12.5	11.3	11.3 to 15	12.6
Betula	45	43.5	45.5	45.5	45.7	43.5 to 45.7	45.1
Carpinus	.....	0.5	1	.....	0.3	0 to 1	0.4
Fagus	0.5	0.5	1	0.5	1	0.5 to 1	0.8
Pinus	30	35	29	32.5	34	29 to 35	32.2
Picea	1	0.5	.....	trace	0.6	0 to 1	0.5
Quercus	7	8	8.5	7.5	5.7	5.7 to 8	7.1
Tilia	1	0.5	0.5	1	0.7	0.5 to 1	0.8
Ulmus	0.5	.....	0.5	0.5	0.7	0 to 0.7	0.5
Corylus	8	7	4	6	5	4 to 8	5.7
No. of pollen grains counted	150	150	200	200	300		

spectra shown in Table I is rather remarkable, as coarse detritus, mainly leaves and stems of *Sphagnum*, was abundant in the

preparations. Much care had to be taken to prevent pollen grains from being omitted or wrongly determined. The time required for each analysis was about 2 hr. In our attempts to get rid of the pollen-obscuring detritus, we proceeded in the following way:

Destruction of the Lignin and Humic Acid Components of the Peat.—In our first experiments we used alkaline oxidative agents, as boiling the peat in the solutions of potassium permanganate, sodium hypochlorite, or hypobromite. Although in some of these experiments a considerable concentration of the pollen was achieved, it was found difficult to avoid some destruction of pollen grains. For further experiments, therefore, an acid oxidizing agent, *viz.*, diaphanol (a solution of chlorine dioxide in acetic acid), was used. This, however, was found to act too slowly and not intensively enough. It was then replaced by a mixture of finely powdered potassium chlorate, acetic acid, and sulphuric acid. This method has the advantage over the diaphanol method in that the amount of the chlorine dioxide liberated by the reaction can be changed within wide limits. The method worked fairly well, but some difficulties were encountered from the slight solubility of the potassium chlorate. The best results were obtained when the potassium chlorate was replaced by the more easily soluble sodium chlorate.

In a Petri dish 0.2 g. standard peat was added to a mixture of 8 cc. glacial acetic acid and 4.5 cc. sodium chlorate solution (100 g. sodium chlorate and 200 cc. distilled water). One cubic centimeter sulphuric acid (80 per cent) was carefully added, drop by drop, and the Petri dish agitated to insure a thorough mixing of the fluids.* The whole was allowed to stand for 12 hr. at laboratory temperature after which the solution was diluted to about 40 cc., and the undissolved material collected by centrifuging. The sediment was washed once or twice in the centrifuge tubes with distilled water and again sedimented by centrifuging, then (to remove the water) washed in the same way twice with acetone and twice with ether (dried over calcium chloride). It was then spread out with a glass rod on the inner wall of the centrifuge tube, and the remaining ether evaporated by short heating on the water bath.

* Great care must be taken in adding the sulphuric acid, otherwise the reaction may become explosive resulting in serious burns to the operator.

Hydrolysis of the Polysaccharide Fraction of the Peat.—The material thus obtained consisted largely of cell walls and was of a faint, yellowish-white color. It was thoroughly mixed with sulphuric acid (1 cc. or less of an 80 per cent solution) and was allowed to stand for 3 hr., after which water was added, and the solid residue collected by centrifuging and thoroughly washed with distilled water as described above. The water was then poured off, and lactophenol (phenol crystals (20 g.), lactic acid (20 g.), glycerin (40 g.), aqua destillata (20 g.) added up to a certain volume, *e.g.*, 2 cc.). Staining was effected by adding a small drop of very dilute methylene blue. The whole was mixed carefully, and a certain quantity, *e.g.*, 0.1 cc., transferred to a counting chamber (Naumann, 1925). Here the pollen grains were counted, and the relative frequencies and the absolute pollen frequency calculated in the ordinary way.

A common blood-corpuscle counting apparatus (Bürker chamber, Thoma chamber, etc.) is too deep to allow a convenient examination of the preparations at the magnifications generally used in pollen statistical investigations. We have therefore devised a special counting chamber (capacity, 0.1 cc.; depth, 0.08 mm.) which is now on the market. It takes even the biggest pollen grains and at the same time allows the use of high-power objectives.

Results of analyses of standard peat treated in the way described above are practically identical with those obtained from peat treated with sodium hydroxide (Table I, page 116).

Everyone working with pollen statistics should have access to reference preparations of pollen grains from recent trees. Such preparations can be made directly from fresh material or from boiling stamens of herbarium specimens with 10 per cent caustic potash and mounting the pollen grains in glycerin jelly. After some practice it is possible even to identify pollen grains of different species within the same genus, for instance, to distinguish the pollen of *Picea canadensis* from the slightly smaller one of *P. mariana*, that of *Pinus Murrayana* from that of *P. Banksiana*, etc. It is often useful to calculate and record in a reference table the limits within which the dimensions of the pollen grains of closely related species vary. In the peat preparations some pollen grains are seen only from above, obliquely, contorted, or even in fragments or, in the case of pine and spruces, as isolated

wings. Consequently, in studying the reference preparations, not only the breadth of the pollen grains should be measured, but also the length and depth of the pollen grain proper, and the breadth, length, and depth of the wings, when these are present. Notes on the finer structure are desirable, also notes on the color, which is variable according to the chemicals used in the preparation of the pollen for microscopical examination.

It is but natural that many difficulties will be encountered by those who venture in the study of pollen statistics. For example, it cannot always be assumed that where there was much pollen there was a correspondingly heavy growth of the trees that produced it—for pollen grains may be carried by the wind for hundreds of miles*—nor can it be assumed that where there are few or none there were few or no trees. If the bog into which the pollen fell was not in proper condition to receive and preserve pollen, it would be lost, and thus no record would remain. Such conditions include a chemical or bacterial state of the water which would cause the pollen grains to decay, a frozen surface over the bog at the time of pollen shedding, or a number of other circumstances.

To get an idea of the manner in which the fossil pollen record ought to be interpreted, samples from the surface of the bog should be examined for pollen grains, and the findings compared with the present distribution and abundance of the forest trees in the neighborhood. For that purpose the forests should be mapped, and their composition ascertained.

An investigation of this kind was carried out by the author of the present chapter in southwestern Sweden in 1920. The surface samples from the district investigated could be classed into two groups according to their pollen content: in the one dominated the pollen of conifers; in the other, that of deciduous trees. The samples of the first group came, as a rule, from the branches of living *Sphagnum* moss; those belonging to the second, from the wet, muddy hollows in the moss carpet. From these observations the question naturally arises: Were these groups of samples fairly contemporaneous in spite of the difference in their pollen content, which could be due to different physical and chemical conditions at the spots from which they were taken; or were they

* For example the pollen of pine and spruce from the continent to Greenland, as shown from analysis of peat from Greenland swamps.

not of the same age? It was inferred that the samples from the hollows were older than those from the sphagnum branches, since the pollen spectra of the former appeared to be about the same as those from samples taken from within the *Sphagnum* peat at about the same levels as the samples from the hollows.

As to the dominance of the coniferous pollen grains in the samples taken from the living *Sphagnum* plants, one could suggest this to be due, at least in part, to the rain waters' having washed the small pollen grains of the deciduous trees farther down into the peat than the larger pollen grains of pine and spruce. That pollen grains are sometimes washed down in this way has been shown by C. Malström of the Forest Service of Sweden. In some laboratory experiments he used pollen of an easily recognizable species, allowing it to shed on the surface of a big sod of peat from a part of Sweden where pollen grains of the type used in the experiment could not be encountered and then pouring water in small portions on the peat. In some of the cases pollen grains impregnated with colloidal gold to assure their recognition when encountered were used for experiments in the field. The results of these experiments indicate that pollen grains are carried down by water *only within the unconsolidated debris* or litter which forms a layer from about 1 in. to 2 ft. thick on the surface of certain types of bogs and that pollen does not penetrate into the true peat. Unconsolidated litter is largely absent from the bogs in southwestern Sweden: accordingly, pollen should not be expected to be carried down into it, in any amount, by water. This was proved by the fact that the pollen flora of the living *Sphagnum* in the bogs was practically identical with that of cushions of *Grimmia* and other mosses growing on rocks and old stumps or other places where the carrying down of pollen grains was impossible.

In the surface samples of *Sphagnum* the frequency of alder and birch pollen seems to bear a fairly close relationship to the actual distribution and abundance of these trees. Both the alder and birch sometimes occur in greater quantities in the bogs than in the surrounding country. This might cause a local overrepresentation of their pollen, making it appear that these species constitute a greater proportion of the forests as a whole than they actually do.

In one instance groves of hornbeam occurred about 100 miles south of the district under examination. Pollen of this species

was not found in the surface samples. It was found, however, fairly regularly, although with a low frequency, not far below the surface in some of the bogs. Hence it might be possible that not very long ago the hornbeam occurred farther north than now.

The frequency of beech pollen was fairly consistent with the recent occurrence of the beech. The same was true of spruce pollen. The spruce avoids the coast, and its range has a well-defined line of demarcation traversing the district, parallel to the coast. The spruce pollen frequency of surface samples from bogs at or near this limit is about 13 per cent; in the inland bogs it is higher; in the coastal bogs, lower.

The frequency of pine pollen varied between 49 and 66 per cent. The pine is a prolific pollen producer, and the pollen is easily carried by the wind and distributed over a wide area. The pine, therefore, is probably overrepresented in most of the pollen spectra. This is largely due to the pine forests in the country at large, as local stands of pine in the bogs seem not to affect the pollen spectra in the same degree as stands of alder and birch.

The pollen frequency of the willows was very low, presumably in accordance with the present distribution of the species belonging to this genus.

The mean oak pollen frequency was only 3 per cent, in striking contrast to the large oak forests of the district. The oak, therefore, had to be considered decidedly underrepresented in most of the pollen spectra. This was true both as compared with the conifers and as compared with some of the other deciduous trees, *i.e.*, the beech, the mean percentage of which is about two-thirds that of the oak, although the absolute frequency of the beech certainly is many times less than that of the oak.

Elm and basswood trees are found scattered all over the district. Only very few pollen grains of these species were seen in the surface samples. In spite of being entomophilous, *Tilia* does not seem to be underrepresented in the pollen diagrams: sometimes its pollen grains are found in great profusion in the peat, which might be due to flowers or whole inflorescences, becoming accidentally embedded in the peat.

The pollen of *Corylus*, or supposedly of this genus, has created quite a sensation during the progress of the pollen-statistical investigations. In certain older strata of many bogs in middle and western Europe it occurs in an abundance sometimes overtopping the total of all the other pollen grains; several theories have been put forward to explain these supposed "hazel forests." The identification of hazel pollen is not always easy: it is very similar to that of the birch, also to that of sweet gale (*Myrica*), which latter, however, is not believed to be subject to preservation in the bogs. Its representation in the surface samples seems to be fairly consistent with the actual frequency of the hazel in the district under consideration.

Then there are some trees and shrubs in the district the pollen of which is not preserved in the bogs, for instance, aspen and several species belonging to the Rosaceae. They are not very prominent in the vegetation, and their absence from the fossil pollen records accordingly is not of much importance.

For the sake of comparison will be added here some observations on the pollen content of the surface samples from a number of bogs or muskegs, made in Alberta, Canada, 1930 to 1931, where, contrary to the conditions of the previous district, trees the pollen of which is not preserved in peat are predominant. The greater part of the area investigated is situated in the wide transition zone, between the forest and the prairies, which stretches across central Alberta. Originally this country was fairly consistently covered by various species of poplar and scrub thickets. The dominant tree is the aspen, and, although much of the land has been cleared for agriculture, probably the major part of the area is still tree-clad. The balsam poplar is locally abundant. Thickets of willows are frequent, and throughout the country may be found *Picea albertiana*, the western form of *Picea canadensis*, occurring singly or in groups. The coniferous covering is greatly extended by the numerous muskegs in certain regions. The chief tree here is the black spruce, although the tamarack frequently accompanies it. Birch is not abundant and occurs chiefly on the sides of some of the valleys and on muskegs. Groves of jack pine, *Pinus Banksiana*, are found in some of the sand-hill areas, whereas the lodge pole pine, *Pinus Murrayana*, is confined to the western extremity of the district close to the Rockies.

Pollen grains of *Populus*, the commonest of all the trees, do not occur in the surface samples. On the other hand, jack pine contributes more than any other tree—about 50 per cent—to the average pollen spectrum of the surface samples, although it has such a local distribution that it might be concealed for weeks to a botanist roaming about in this vast district. Tamarack pollen has not been found, or only dubitably so, although there are hardly any muskegs without tamarack.

The birch pollen sometimes attains a frequency of 25 per cent or thereabout, owing to local overrepresentation caused by the pollen of *Betula glandulosa*. The pollen of the white spruce, the climax tree of the country, has a low frequency—up to 14 per cent. The frequency of the pollen of the black spruce is very variable: the highest percentages—about 50—were found in muskegs with a dense growth of black spruce.

The alders are decidedly less common than the willows, but their pollen, nevertheless, has nearly the same frequency as that of the willows or about 6 per cent. Out of 1,500 pollen grains counted, just 1 came from the hazel. The hazel seems to be fairly common, although it never appears in quantity.

We can infer from these observations that, if conclusions as to the composition of the present forests in Alberta should be drawn in the customary way of drawing conclusions regarding the forests of the past, speaking of a "birch time," a "pine time," a "spruce time," etc., on the basis of pollen statistics only, they would turn out to be entirely misleading. There is not a pine time in central Alberta at present, although the average pollen spectrum of the surface samples is that of a pine time. Moreover, in the surface samples there is no record of the dominating trees aspen and poplar, and likewise none, or only a scanty one, of such widely distributed trees as *Larix* and *Corylus*.

This shows that the problem essential to a successful start of pollen statistical investigations is one of ascertaining the composition of the present forests and of studying the processes connected with the catching and preservation of the pollen grains in peat and muck under formation (the "Actuopaleontology" of the bogs).

In the pollen statistical literature the term "pollen frequency" is often met with. Pollen frequency means the number of pollen grains per square centimeter of a preparation. The pollen frequency is by no means always proportional to the density of the forests that produced the pollen. A peat formed slowly would have a greater pollen frequency than one formed quickly, provided they both had the same capacity for catching and preserving pollen grains. In addition, a low pollen frequency might be encountered in peat from a well-wooded country, if the bog surfaces at the time of pollen shedding were not in proper condition to catch and preserve the pollen; and a relatively high pollen frequency could be found in bogs in districts void of forests if their surfaces could catch and preserve the pollen drifted there from a distance. But while it is realized that the pollen frequency figures are not direct indices and, unless properly interpreted, may even be misleading regarding the composition and density of the forests, it is believed that such figures, if calculated from a great number of localities in different countries and then compared with each other, could yield results that would show lines along which further research bearing on the theory of pollen statistics could be done.

The difference between the pollen frequency of bogs in the small and rather far-off Scottish islands and that of some high moors in northwestern Germany is very great, the figure from the Shetland Islands being about 8; that from Germany, about 330. The highest pollen frequency so far met with in Alberta was 90 (from bogs in the coniferous forests near the foothills of the mountains); the lowest, 14 (from a bog in the park land not far from the prairie). The highest pollen frequency from Alberta is three times less than that of some bogs near Achnasheen in the poorly wooded Rossshire in northern Scotland, and the lowest is about the same as that from the Orkney Islands, which are now practically treeless. The pollen frequency of the bogs in Alberta is thus on the whole strikingly low.

To get a clue to the explanation of this fact we must consider that "dead" bogs, *i.e.*, those where peat no longer forms, cannot catch and preserve pollen grains. This is also the case with living bogs during their inactive period, when the cold of the winter, exceptional drought, or, in some types of peat deposits, excessive rain puts a temporary stop to the formation of peat. As to the bogs in Alberta, long, cold winters and rainless springs would tend to retard the annual peat-forming activities to a considerable extent. Thus, for instance, the spring of 1931 was unusually dry in the district of Edmonton, and in the latter part of April and the first weeks of May when many trees and shrubs such as hazel, alder, birch, and poplar, were in bloom,

the ground was still covered with yesteryear's dead plant remains, and hardly any green growth was in evidence. Certainly no formation of peat was going on at that time. Thus it might be fair to assume that the comparatively low pollen frequency of the bogs in Alberta is due to their low power of catching and preserving pollen grains, probably less than that of the bogs of oceanic western Europe, where the dead season of the bogs is shorter or, in some cases, possibly altogether absent.

The low pollen frequency in Alberta might be explained by the fact that, in spite of the short summers, peat is formed there very rapidly in most places; and trees, the pollen of which is not preserved in the bogs, play a much more important role in Alberta than in most parts of Europe. It is rather enticing to see the matter in this way; but then there is still to be explained the comparatively low pollen frequency of the bogs in the dense pine forests near the foothills of the Rocky Mountains, to which such an explanation could scarcely apply.

To ascertain the problems involved and their importance it is necessary to make experiments, chiefly in the field, extending over several years. Only in this way can knowledge of the actuopalaeontological processes be substantially promoted, and a more reliable key for unlocking of the palaeobiological problems be provided. This is urgently needed, as there is a tendency with many of those carrying out pollen statistical research to seek more and more distant objects for their studies, distant with regard both to time and to space. If the knowledge of the actuopalaeontological processes is not duly broadened at the same time as the pollen statistical research penetrates to such faraway regions, this kind of research will undoubtedly provide results of only illusory value.*

* For a bibliography of pollen statistics the reader is referred to Erdtman (1927, 1930, 1932, 1934*b*), and for a further discussion of methods to Kräusel (1929) and Erdtman (1933, 1934*a*).

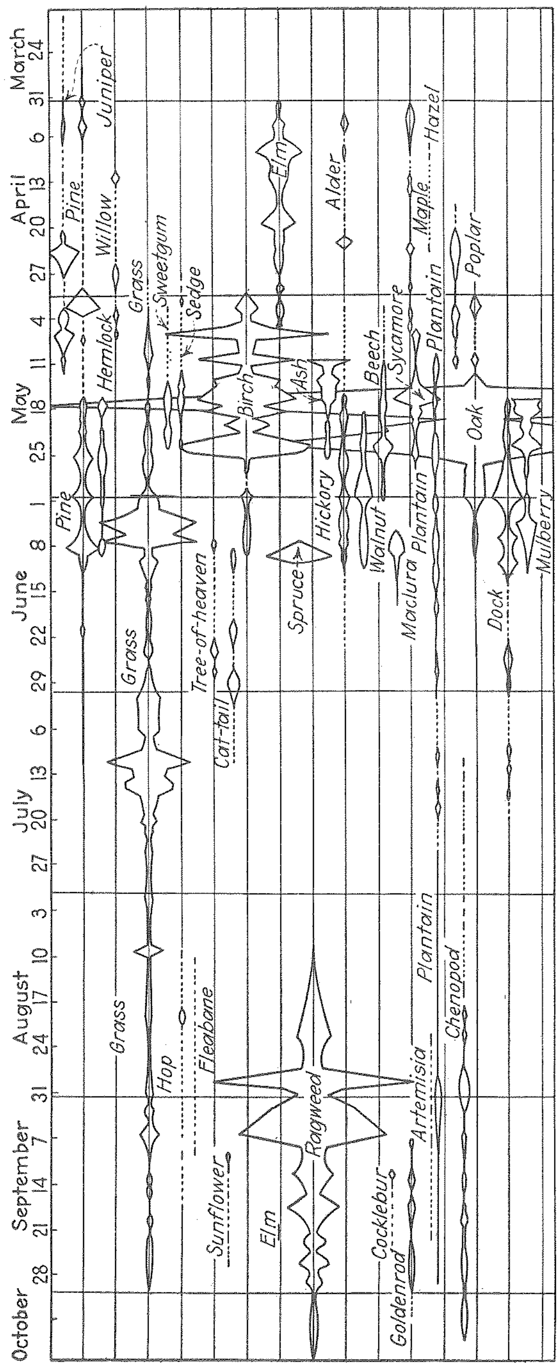

Chart I.—Record of the atmospheric pollen in the city of Yonkers, N. Y., during the year of 1932. The horizontal distance between the vertical lines represents 50 grains of pollen caught on an area of 3.5 sq. cm. during 24 hours. The graphs record the daily pollen counts, somewhat rounded out to eliminate the daily fluctuations.

CHAPTER V

ATMOSPHERIC POLLEN

A study of the atmospheric pollen forms an essential part of all pollen surveys. A single season's record of the succession of species of pollens which invade the atmosphere of any given locality furnishes valuable information regarding its hayfever potentialities. Each year the cycle faithfully repeats, with only such minor fluctuations as may be due to seasonal variations. Consequently, information gained in this way is of much more direct application to the handling of hayfever patients than even the most exhaustive botanical studies. Every community, therefore, should have its seasonal atmospheric pollen accurately determined and recorded in a readily available form.

The accompanying chart is a record of the atmospheric pollen for the city of Yonkers, New York, during the summer of 1932. This record is essentially the same as those of 1930 and 1931, except that the pollen of the early flowering trees reached their maxima a little earlier in 1931 than in 1932. For example, the deluge of oak pollen, always a conspicuous feature in these charts, took place on May 11 and 12 in 1931 instead of May 16 and 17, which is the time it occurred in 1932; also it was a little heavier in the latter year. As a rule, however, it is only among the early flowering trees that even such deviations as this in the time of pollination occur.

The pollen was caught from the air by exposing microscope slides coated with glycerin jelly, as already described.

The building where the slides were exposed is five stories high and stands on a slight eminence near the business center of the city. This elevation was chosen to gain general average conditions by avoiding the effect of the immediately local vegetation. It perhaps accounts for the relatively low grass count; for the grains of most kinds of grass are rather large, distinctly larger and less buoyant than those of the pollen of such plants as the ragweeds. However, this is perhaps less of a disadvantage than having the count overbalanced by the pollen from the plants of the immediate vicinity, as would be the case nearer to the ground level. Even at this elevation above the ground the immediately local flora is reflected to some extent in the pollen count. For example, several large trees of both Osage orange and mulberry are growing in the grounds of the laboratory. As a consequence, the pollen counts of both of these are obviously unduly high. Throughout the rest of the region these two species are relatively rare—much less abundant than sweet gum, for example, which their pollen counts outnumber on the chart.

The city of Yonkers is situated in the Hudson River valley, virtually at sea level. To the north and east, beyond the limits of the city, the country is partly built up and partly wild. The suburban sections are nicely cultivated, but the greater portion of the rest of the area is badly or not at all cultivated and is fully invaded by a diversified weed flora. The entire region appears to have been in the past a mixed forest of oaks. Of course, the majority of the trees have been destroyed, but many remain, some of great size and age. The commonest species of the oaks which remain are black, white, chestnut, scarlet, and swamp white. To the southeast and south lie the great metropolis of New York and its suburbs, covering a region of which the vegetation is for the most part reduced to a minimum. To the west lies the Hudson River, and on its opposite bank, which is the New Jersey shore, about a mile from the city, stand the Palisades, rising 500 ft. above the level of the river. Their tops are heavily wooded with a young second-growth mixed forest which extends westward only a short distance to be replaced by improperly cultivated farm land, harboring many weeds and extending many miles westward. To the south of the Palisades, in a southwesterly direction from the city, lie the famous New Jersey meadows. It appears that much of the pollen caught on the slides originated in these adjacent parts of New Jersey, because the count has a tendency to rise abruptly as the wind swings into a westerly direction. And the New Jersey meadows appear to have been the only possible source of the cattail pollen, which is that of *Typha angustifolia*, a species which, while abundant in the New Jersey meadows, is rare on the New York side of the river.

The first grains to be caught on the slides in the springtime were those of juniper. The different species of juniper cannot be distinguished in their pollen, but *Juniperus virginiana* is the only one common enough in the vicinity to produce the pollen recorded. The curious fluctuations represented in the bulges of the curve of this species on April 5 and 23 and May 6 are due to weather fluctuations. This is indicated by the fact that similar fluctuations occur in the curves of one or more of elm, alder, hazel, maple, poplar, and birch at the same times.

Birch pollen is represented throughout the month of May, extending even a little into June. Three species of birch are common in the vicinity of Yonkers—white, black, and yellow. Their pollens can be distinguished from each other only upon careful and minute examination of each grain, a task which is scarcely feasible in making pollen counts. The different species of birch pollinate in the succession named, generally with considerable overlapping. The first two bulges in the birch graph represent the flowering of the white birch, *Betula populifolia.* The third bulge, with its minor fluctuations which are due to weather variations, represents the flowering of the black birch, *B. lenta;* and the fourth represents the yellow birch, *B. lutea.*

Oak pollen began to be caught about the first of May and reached a count of about 20 grains on the second. Thereafter only occasional grains were caught, until May 16 and 17 when the count suddenly increased to 1,034 and 1,308, respectively, after which it dropped off considerably but rose again on May 23 to 644. These two major bulges in the graph repeat themselves from year to year and are undoubtedly due to different groups of species. At the time of the oak-pollen maxima the atmosphere is actually hazy with oak pollen. Nevertheless, in spite of its great abundance, which outnumbers all other species, oak pollen causes relatively little hayfever in the region.

In recording the pollen counts of the different grasses no attempt was made to distinguish the grains of the different species by their microscopic characters. But the different species can be separated out to some extent in the graph by taking into account their flowering periods. The first grains of grass appear toward the end of April, when the only species flowering was low spear grass, *Poa annua.* The count thereafter soon increased, reaching about 20 on May 8, as sweet vernal grass, *Anthoxanthum adoratum*, came into bloom. Toward the end of May, June grass, *P. pratensis*, quickly followed by orchard grass, *Dactylis glomerata*, began to flower. The maximum of their flowering is represented by the double-pointed bulge in the grass-pollen graph of June 4 and 7, when the counts reached about 140. As those two species completed their flowering, the grass-pollen count fell rapidly and continued low until the flowering period of timothy, *Phleum pratense,* which began about the end of June. This was soon followed by redtop, *Agrostis palustris,* and the bent grasses, *A. tenuis,* etc., whose maximum flowering period is reflected in the bulge of the grass-pollen graph occurring between July 11 and 15. This graph shows that timothy, in spite of the great emphasis that has been laid upon it in most hayfever literature, stands no better than fourth in the list of grasses for Yonkers; its pollen production is considerably less than that of June grass, orchard grass, and redtop. After the cessation of flowering of redtop and the bent grasses, with which early summer hayfever in Yonkers virtually ends, the grass count continued low, with a minor unexplained increase on Aug. 9, until the beginning of September when crab grass, *Syntherisma sanguinalis,* and goose grass, *Dactyloctinium aegyptium,* came into flower, causing the grass count to show a noticeable increase which reached a maximum on Sept. 6. After this final flare-up grass pollen continued to be caught, only two or three grains at a time, until the end of the season.

The first grain of ragweed, which proved upon examination to be of the giant ragweed, *Ambrosia trifida,* was caught on Aug. 8, only a few days after the first plants of that species were observed in flower. For about the first week thereafter the ragweed pollen caught proved to be entirely or predominantly of the giant species. But as the count increased, pollen of the dwarf species, *A. elatior,* was added, and throughout the greater part of the season the pollen of the two species appeared to be represented by about equal numbers of grains. The combined ragweed count gained rather steadily, reaching its maximum on Aug. 29. The diminution shown in the graph between Aug. 22 and 27 was due to unfavorable weather conditions, which were likewise reflected in the graphs of all other species recorded during that time. The sudden diminution occurring in the ragweed count on Sept. 1 is unexplained. It is apparently not due to unfavorable weather conditions, since it is accompanied by an increase in the pollen count of the other species. With other minor fluctuations the

pollen of the ragweeds continued with dwindling count until well into October. In the Yonkers region the late summer hayfever, which is due almost entirely to the two ragweeds, starts between Aug. 15 and 20 and usually stops toward the end of September or early in October, and throughout the season its severity runs closely parallel with the pollen count of the two ragweeds.

Pine.—It is not possible to distinguish the different species by their pollen. Undoubtedly several are represented in the graph. The bulk of the pollen caught toward the end of May and the beginning of June corresponds to the flowering period of Austrian pine, *Pinus nigra* var. *austriaca,* which is extensively cultivated in and about Yonkers.

Hemlock, *Tsuga canadensis.*—Trees are scarce within the city limits but are fairly abundant outside.

Spruce.—Species unknown, but its pollen graph, extending over only 3 days, corresponds to the flowering of *Picea rubens.*

Sedge.—The different species are not easily distinguished in their pollen, but the first few grains caught in April and May correspond, in point of time, to the flowering period of *Carex pennsylvanica,* while those caught later in May correspond to *C. stricta.*

Willow.—The first grains caught correspond, in point of time, to the flowering period of pussy willow, *Salix discolor,* and the latter to crackle willow, *S. fragilis.*

Elm, *Ulmus americana,* is exceedingly abundant throughout the region. Several other species also occur sparingly, but no attempt was made to distinguish their pollen from each other. A single grain of elm pollen was caught on the slide late in September. This may have been from a tree of *Ulmus serotina,* a late-flowering species native of the south but occasionally planted northward.

Alder, *Alnus incana,* is abundant throughout the region.

Hazel.—The two native species *Corylus americana* and *C. rostrata,* and the European species *C. Avellana* occur sporadically throughout the region, but their pollen grains are not distinguishable from each other.

Maple.—The pollen grains of the different species are not distinguishable from each other; but the first occurrence of maple pollen on the slides corresponded to the flowering of silver maple, *Acer saccharinum,* which is much planted in and about the city and flowers ahead of all the others. It is followed by red maple, *A. rubrum;* sugar maple, *A. saccharum;* sycamore maple, *A. Pseudoplatanus;* and Norway maple, *A. platanoides*, which probably all contributed to the maple-pollen count recorded in the graph.

Poplar.—The species of poplar were not determined. *Populus Eugenei* is the most abundant species within the city limits, but, being an artificially produced hybrid, it sheds but little pollen, and this mostly abortive, consisting of misshapen grains and empty skins. A part of the pollen caught answered this description. Beyond the limits of the city *P. deltoides* and *P. grandidentata* are abundant, and it is probably from them that most of the poplar pollen was derived.

Sycamore, *Platanus occidentalis*, is common throughout the region; two trees of this species grow within the laboratory grounds.

Ash, *Fraxinus americana*, is abundant throughout the region.

Beech.—The different species of beech pollen were not distinguished, but the long period over which beech pollen was caught suggests that more than one species was involved. Both the native species, *Fagus grandifolia*, and the introduced European species, *F. sylvatica*, in several varieties, are abundant.

Sweet gum, *Liquidambar Styraciflua*, is exceedingly abundant throughout the region.

Walnut.—Both the black walnut, *Juglans nigra*, and the butternut, *Juglans cinerea*, are found sparingly throughout the region. Their grains can be distinguished from each other but were not separated in making the counts, though it was observed that both occurred.

Hickory.—The pollen grains of the local species of hickory cannot be readily distinguished from each other. *Carya ovata*, *C. alba*, *C. glabra*, and *C. cordiformis* are found in the region.

Tree of heaven, *Ailanthus glandulosa*, an Asiatic introduction, is abundant in the region.

Plantain.—The species recorded in the graph is entirely English plantain, *Plantago lanceolata*. Grains of the two other species (*Plantago major* and *Plantago Rugelii*), which grow commonly in the region, were only occasionally caught on the slides, a grain or two at a time. The pollens of these three species are easily distinguished from each other.

Chenopod.—Under this name are included the pollen of lamb's-quarters, *Chenopodium album;* Mexican tea, *C. ambrosioides;* and the pigweeds, *Amaranthus retroflexus* and *A. hybridus*. All four are common, and their pollen cannot be separated with certainty.

Artemisia, *Artemisia Absinthium*, is a recent introduction, adventive from Europe.

Goldenrod.—The different species of goldenrod cannot be distinguished in their pollen. Many species occur in the region, some of them in great abundance, but one species, *Solidago speciosa*, greatly outranks all others in its production of pollen.

Sunflower.—The fact that sunflower, *Helianthus annuus*, is cultivated in considerable quantities in the grounds of the laboratory probably explains the presence of sunflower pollen on the slides.

Fleabane.—*Erigeron canadensis* and *E. strigosus* are exceedingly common. Their pollen grains, however, are not distinguishable from each other.

Hop, *Humulus Lupulus*, occurs sporadically and grows sparingly in the laboratory grounds.

Linden, *Tilia americana*, occurs in several varieties. Though insect pollinated, its pollen was caught sparingly from June 22 to 30.

Besides the pollen species which are shown in the graph, a few grains of rush, wild cherry, privet, and sumac and the spores of horsetail, several ferns, and many fungi, including *Alternaria*, were caught.

After a hayfever patient has been properly typed, a glance at a chart of this kind will tell him whether or not, or just when, he is likely to have hayfever in the locality for which the chart has been prepared. It is therefore imperative that every locality, especially those that cater to the tourist traffic, and hayfever resorts in particular, should have such charts available for the inspection of prospective visitors who may be hayfever sufferers or for physicians who wish to learn the most favorable places to which to direct their patients.