THE FLOWERING PROCESS

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Frank B. Salisbury

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FRANK B. SALISBURY
Professor of Plant Physiology
Colorado State University

PERGAMON PRESS
OXFORD · LONDON · NEW YORK · PARIS
1963

PREFACE

THE principal purpose of this book is to discuss in its biological framework, the conversion from the vegetative to the reproductive state in higher plants. There are two aspects to the study of this conversion; first, the changes within the plant which lead to the conversion, and second, the conversion itself. The first of these aspects has been studied most and is emphasized.

The extent of scientific development in this field is quite amazing. Probably only a small portion of the world's population is aware of this rather isolated branch of science, but a complete collection of papers relating to the physiology of flowering would fill a rather impressive bookshelf. It would be fairly easy to find 1000 such papers. Thus a straightforward complete review of this work would probably result in a very thick volume. Luckily, space allotments from the Publisher saved the author's time and patience from being put to such a test. A complete summary volume would be of unquestioned reference value to science, but unless the author were gifted, such a condensed recounting of experimental work would quite probably make for very dry reading. All of this poses a dilemma for an author: he can try to cover the field and probably lose his reader in the mass of conflicting and often unrelated facts, or he can concentrate on certain aspects of the physiology of flowering and thereby slight other aspects which may be equally interesting and important. I decided on the second approach.

The book is addressed to graduate students and others who might be interested in the topic presented approximately at the graduate level. It is assumed that the reader has a good background in some phase of biology but that his acquaintance with the physiology of flowering is rather cursory. It was my intention to discuss broad aspects of the topic in the first four chapters, but in some respects these became as specific as the ones which follow. I feel that they will provide a good introduction to the last part of the book for the student who already has some knowledge about the flowering process, but the real beginner might want to return to Chapters 2, 3, and 4 after the other chapters have been completed.

In the last six chapters I gave in without reservation to the temptation to discuss in some detail my own main interest, relying heavily upon personal research experience. This interest is in the sequence of biochemical and biophysical events which take place within the plant, beginning first with response to the environmental stimulus imparted by the relative length of day and night and culminating in the production of flowers by the plant primary shoot meristems. Although many species are mentioned, the theme of the narrative always centers around the cocklebur.

This is not because this plant is highly "typical". The converse is probably true, and the principal atypical response of this plant, flowering after exposure to a single long dark period, makes it an ideal research plant. Thus my preoccupation with this species as a "type", even though it is not exactly "typical", is based upon its nature as an experimental object. This nature readily allows the experimenter to think of the flowering process as a series of catenary events, each bearing some time relationship to the single "inductive" dark period. Other plants are now known to be equally well suited, but our experience with them is not yet so extensive as that with the cocklebur.

In an early version of the manuscript, the book was addressed largely to high school teachers of biology. There was one aspect of this early approach which appealed to me very much: the flowering process is a fairly good summary of biology in general. This is discussed briefly in Chapter 1, and it is hoped that the idea is evident throughout the book. The breadth of such an isolated topic is quite impressive, and this breadth must surely be typical of what one might find upon intensive study in virtually any "narrow" field. There is a unity in science, and the specialist who would really specialize will find more and more that he must be a general practitioner.

Since it seemed desirable to avoid the style of a literature review, an effort was made to reduce the number of references in the text to a bare minimum. This is possible only because a number of excellent reviews have been written in recent years. These are listed in the bibliography, and section headings often refer the reader to a number of them. Such references in section headings were chosen according to my impressions about the reviews with which I am most familiar.

Many of the books and reviews are at least as broad as the present volume and could be used as references in virtually all sections. The interested reader who wants to see original papers can find references according to topic in nearly any of these recent books or reviews (see especially 3, 9, 11, 14, 20, 21, 22, 25, 26, 28, or 32). Actually, nearly all work not directly cited in this book is documented in my own review (32). The printed report of the recent symposium in Australia will contain very recent references, and I am indebted to Dr. Erwin Bünning for showing me some of these manuscripts. My last revision was strongly influenced by them.

In spite of this approach to literature citation through reviews, it was felt that more direct reference should be made in cases where work (rather than well-known conclusions) is specifically mentioned, using the name and location of the investigator. In these cases a recent pertinent paper is cited. Figures copied from published papers are also acknowledged in the figure captions, giving further specific references. Table 7–1 contains references to a number of papers which are either quite recent or not easily found in the reviews. There is also a considerable amount of unpublished work which is discussed in the book. Usually this is apparent from the figure headings.

The manuscript was used in an early duplicated draft in an advanced plant physiology class at Colorado State University during the spring of 1962. As a result of discussions in this class, many of the ideas now incorporated into the text developed, and a number of experiments were performed. Thus I am indebted for both intellectual and material help to the members of this class: Charles Curtis, Lee Eddleman, Nagah Karamani El Sayed, James Gary Holway, Deogratias Lwehabura, Oscur Schmunk, and James Whitmore.

After arriving in Tübingen (in August, 1962, for a sabbatical year), the manuscript was almost completely rewritten. Drs. Arthur Galston, Anton Lang, Jan Zeevaart, and Phillip Wareing had read the duplicated version, and their comments contributed much to the rewriting. Drs. Erwin Bünning and Lars Lörcher also read parts of the manuscript and made valuable suggestions. During the rewriting, Drs. Galston and Wareing supplied immeasurable help by reading and commenting on the Tübingen version. I am also deeply indebted to my assistant, Jean Livingston, and my graduate student Carol Pollard, who answered my many mailed requests to Colorado, sometimes by performing experiments to answer questions that kept coming up. My wife, Marilyn, was indispensable during this period, since she typed the rewritten manuscript to send to Drs. Galston and Wareing.

The following secretary-technicians have helped with clerical and experimental aspects of our cocklebur research in Colorado since 1955: Pauline Christiansen, Anita Brooks, Joan Maxwell, Annette Hullinger, Marjorie Smith, Katherine Kline, Marilyn Young, and Sandra Howard (who typed one complete version of the manuscript). My colleagues in the Department of Botany and Plant Pathology, especially Dr. Cleon Ross who works on biochemical aspects of cockleburology, have been helpful in many ways. Dr. Ross read and commented on the final manuscript. My graduate students have contributed materially to the original work, some of them (Walter Collins, Leona Harrison, and Carol Pollard) by dissertation work on the physiology of flowering, and all of them (Edward Olsson, Robert Mellor, Merrill Ross, and George Spomer) by ungrudgingly helping with all-night experiments.

I would especially like to express appreciation to Colorado State University, the National Science Foundation, and the National Institutes of Health for providing facilities and financial support for our cocklebur research and for preparation of this manuscript.

FRANK B. SALISBURY.

Botanisches Institut
Tübingen, West Germany

Note — Although decimal points throughout this book are given according to American practice (and also the spelling, since the author is a U.S. citizen), on several of the diagrams raised points appear in the decimals. It is hoped that this small inconsistency will not mislead the reader.

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 and 5 Fitzroy Square, London, W.1

PERGAMON PRESS INC.
122 East 55th Street, New York 22, N.Y.

GAUTHIER-VILLARS ED.
55 Quai des Grands-Augustins, Paris, 6e

PERGAMON PRESS G.m.b.H.
Kaiserstrasse 75, Frankfurt am Main

Distributed in the Western Hemisphere by
THE MACMILLAN COMPANY · NEW YORK
pursuant to a special arrangement with
Pergamon Press Inc.

Library of Congress Catalog Card Number 63-19739

Set in Monotype Times New Roman 10 on 12 pt.
and printed in Great Britain by
THE BAY TREE PRESS, STEVENAGE, HERTS.

CONTENTS

CHAPTER 1

FLOWERING IN ITS BIOLOGICAL FRAMEWORK

SOME of the most general, and indeed the most exciting aspects of biology are an integral part of the flowering process, and most of the basic fields are represented to a greater or lesser degree. Any study of plant or animal function is physiology, and so the discussions to follow will emphasize physiology. Of course any function is dependent upon some entity or structure, and in the study of flowering we are concerned with the origin of structure itself. Thus we approach the fields of anatomy or morphology. Many plants flower in response to some change in the environment, so the topic bears a valid relationship to ecology. Different kinds of plants respond in different ways, and as one tries to organize these responses according to type, one does work not too unrelated to that of the taxonomist. The flowering response is inherited, and it is possible to study its genetics; indeed, flowering involves the response of the genes and their products to the environment, and study of such things lies in the new field of molecular biology. If there were space, one could also discuss certain applied aspects of flowering in the fields of horticulture and agronomy. Obviously, if flowering could be controlled, agriculture could be revolutionized.

It is amazing how a study in depth of any topic in biology may cut across nearly the entire field of biology itself. The process of flowering is certainly no exception, although there are aspects which are not encountered, such as nerve or muscle function. Certain rather unlikely subjects such as paleobotany or evolutionary mechanisms do bear a relationship to flowering, although we will not have much to say about them here.

We will not approach the flowering process by studying its relationship to each of the traditional fields listed above. Rather, we will keep in mind five general biological areas:

1. Diversity and Uniformity of Biological Material

2. Response of an Organism to its Environment
3. Biological Timing
4. Biochemistry
5. Morphogenesis or the Origin of Form

A brief discussion of these five topics now will serve as an introduction to the more detailed discussion of the flowering process which follows. We think of flowering in terms of component steps or events which are taking place within the plant and which ultimately lead to the formation and development of flowers. The whole point of the first topic is that these steps may vary considerably from one species to another. Thus in discussing the last four topics (and in the last six chapters of this book) we shall consider the steps primarily as they are thought to occur in our "type" plant, the cocklebur, although deviations will often be mentioned.

1. *Diversity and Uniformity of Biological Material*

In considering this topic one cannot help feeling somewhat like a pendulum. It is quite obvious that the world of living things consists of a myriad of diverse forms. The list of known species extends into the millions and the diversity is enormous. Consider the protozoa, jelly fish, sponges, flat, round, and segmented worms, starfish, shell fish, snails, shrimps, finned fish, lizards, birds, mammals, and all the other sundry groups of animals. Then think of the bacteria, many kinds of algae, fungi, mosses, liverworts, ferns, conifers, and flowering plants. The taxonomist estimates that we shall one day know three to five million kinds of insects alone. Thus on one swing of the pendulum we are fully aware that there are many kinds of living things.

Yet the significant generalization of modern biology is that all of these various organisms have a number of important and basic functions in common. This is most striking when one considers the biochemistry of the cell. Respiration, for example, proceeds along essentially the same metabolic pathways in all living things, and this also seems to be true for many other processes such as protein synthesis, fat metabolism, etc. So the other end of the pendulum's swing is the concept that living things are really all very much alike. Is this true in the flowering process? At this stage of the game we simply do not know. Some workers have assumed that it was true —

that the flowering process is essentially the same in all flowering plants with only slight modifications which apparently lead to a diversity of response. In my opinion it is too soon to draw this conclusion. It has in the past led to application of findings obtained with one plant to understanding of flowering in another — and subsequent work has frequently failed to support this.

We shall see in the next chapter that the diversity of response in flowering is very great. If we want to make the classification scheme complex enough, we can probably produce a separate category for each species or variety. In many cases these differences are quite striking. A short-day plant is inhibited in its flowering by a brief light interruption of the dark period. A long-day plant is promoted in its flowering by the identical treatment. In one short-day plant far-red light is without effect (or promotes) during the dark period; in another it inhibits flowering. The pendulum should be allowed to swing far to the diversity side, and Chapter 2 is written to try to push it far in that direction.

But it must also be allowed to swing back to the uniformity side. If there is any sort of natural relationship among the flowering plants, as modern biochemistry implies, why shouldn't there be some basic, common underlying mechanisms in the flowering process? There are at least two excellent reasons to think that this is the case. The pigment system which switches the plant's metabolism from the light to the dark status seems to be common to all higher plants — certainly to the ones which we will be discussing. Furthermore, there is evidence from grafting experiments that the flowering hormone itself is the same in species and varieties which in other respects show opposite responses.

Is the apparent diversity of response really only a matter of slight modification of a common basic mechanism? Or have the modifications become so extensive that we should not think in terms of a single mechanism but rather of a number of fundamentally different mechanisms which do happen to be similar in certain respects? Much more research is required before these questions can be answered, and so for the present we can only let the pendulum swing freely while we wait for the facts to come in. The situation is, at any rate, common to most of biology. We are impressed by the uniformity, but the diversity is becoming more and more interesting.

FIGURE 1–1

In order to show the basic response of a number of species to day-length, seeds were planted in the spring of 1962 by Mohamed N. K. El Sayed of the advanced plant physiology class at Colorado State University, and half of the plants of each species were placed under a light-proof box every day at approximately 4.00 p.m. and removed the following morning around 8.00 a.m., while the remaining plants were left under the long-day conditions of our cocklebur greenhouse (about 20 hr of light—see Chapter 5). At various times after planting, as indicated by figures in each picture, the plants were photographed. Scientific names are given in the appendix. Figure A is an example of a day-neutral plant; Figs B to F are absolute short-day plants; Fig. G is nearly an absolute short-day plant, although flowers can also be seen under long-day conditions occasionally; Fig. H is at best only a quantitative short-day plant (see Chapter 2), since it flowers on both long and short days, but faster on short days; and Figs. I to L are absolute long-day plants. Note in most photographs the strong effects of day-length upon vegetative growth as well as flowering. In many cases, exposure of the plants to the day-length which causes flowering from the time they first emerge as seedlings produces flowers on such small plants that the resulting examples are not very typical of flowering plants in nature (e.g. Figs. B, C, D, F, and K). Thus in Figs. E and I, plants were held under non-inductive conditions for a few weeks before they were induced to flower.

2. *The Response of an Organism to its Environment*

In our "type" plant, the cocklebur, the fundamental response to environment is a response of the leaf to an uninterrupted dark period which exceeds about 8 hr 20 min. Given such a dark period, the plant flowers; on shorter dark periods the plant remains vegetative. Of course there are other effects of environment: temperature must be right, adequate soil nutrients and water must be available, and if the dark period is to be highly effective, it must be preceded and followed by exposure of the plant to high intensity light. Obviously the response to environment is a matter of physiology, but it can nevertheless be considered in an ecological sense (see Chapter 3).

The whole modern study of the flowering process was initiated quite recently (1920) by the discovery that flowering in many plants is an environmentally conditioned response. W. W. Garner and H. A. Allard, working at the United States Department of Agriculture Plant Industry Station at Beltsville, Maryland, wondered about the peculiar flowering habits of two economically important species. A variety of tobacco, called Maryland Mammoth, grew 10 ft tall during the summer months at Beltsville, but failed to flower and set seed. Transplanted as cuttings or root-stocks into the greenhouse, plants would flower in winter when they were less than 5 ft tall. A certain variety of soybean, when planted successively at various times throughout the spring, tended to come into flower on the same summer date regardless of the planting time. This variety (and some others as well) would flower in winter in the greenhouse even when the plants were very small. Obviously there was something about winter greenhouse conditions which seemed to cause flowering in these two species.

Garner and Allard first tested effects of light intensity, temperature, and available soil moisture and found no definite effect on flowering. Then, almost reluctantly, they tested the effects of day-length, by extending it with artificial light or shortening it by placing plants in cabinets. They were thus able to show that flowering occurred in these two plants when days were shortened — regardless of other environmental conditions (providing it was not too hot, dry or shady for survival). They called the class of plants that responds in this way short-day plants. With other species, long days resulted in flowering (long-day plants), while flowering occurred in some plants on any day-length (day-neutral plants). This basic response is illustrated, with some plants commonly used in such studies, in the photographs of Fig. 1–1. Garner and Allard called their newly discovered phenomenon *photoperiodism*. In later experiments of these and other workers, it was found that many plants respond more to the night-length than to the day-length; thus the term *photo*-periodism is not entirely accurate, but usage has made it secure.

It was known by nineteenth century farmers in the United States that winter wheat, which usually flowers in the spring after being planted the previous fall, will flower even if it is planted in the spring, providing that moist seeds have been exposed to low temperatures for a few weeks. This flowering response was also studied intensively in the years following 1920, primarily in Europe and Russia, but to some extent in the United States (see Chapter 4).

Thus it became clear that the change from the vegetative to the reproductive condition in higher plants may often be initiated by some change in the environment. The changing length of day seems to be such an obvious aspect of this environment that it is indeed quite surprising that the discovery was made virtually within our own generation.

3. *Biological Timing*

Perhaps the most impressive thing about the phenomenon of photoperiodism is the implication that the organism is measuring time (see Chapter 8). Thus the cocklebur requires at least 8½ hr of uninterrupted darkness (the so-called critical dark period or critical night) to initiate flowers. Most amazing of all, essentially the same critical dark period is required over at least the temperature range of 15 to 30°C. We can easily visualize time measurement by thinking of the time required for completion of a chemical reaction, but that this should be independent of temperature is not easy to understand.

It is probably safe to say that the formal study of biological timing was first initiated by Garner and Allard in 1920, although previous work was closely related, and man has always had a sense of time and probably suspected that other animals, at least, shared this. In the late 1920's zoologists observed that bees could be trained to feed at certain times of day. About this time rhythmical movements of leaves and other organs were clearly shown to continue under constant environmental conditions. In spite of this early work, the idea of biological timing did not occupy the minds of many biologists until the 1950's.

It is now known that this phenomenon might be a general manifestation of virtually all living things. This cannot be stated as yet with absolute certainty, but nearly all lines of evidence seem to converge on this generalization. As we shall see in Chapter 8, photoperiodism is only one of many examples of biological time measurement. It has become a problem of fundamental importance, and obviously the flowering process is an excellent example.

4. *Biochemistry*

Following time measurement (critical night), a flowering hormone seems to be synthesized in the cocklebur leaf. Energy and proper substrates are required. As mentioned above, the dark period is ineffective unless it is preceded by a period of high intensity light — a period of photosynthesis. The response to the light environment also is biochemistry, since it is mediated through a pigment system, although we might refer to this process as *photo*-biochemistry (a subdivision, perhaps, of photochemistry).

The light response is a most interesting process from the biochemist's viewpoint, since it apparently involves a trigger type reaction. In photosynthesis the absorbed light energy is converted to chemical bond energy, but in flowering the quantity of light energy involved is extremely small, and rather than itself *causing* flowering to occur, it turns the switch which then influences the biochemistry of the flowering process. The remarkable fact is, that in the case of the cocklebur or other short-day plants, turning the switch during the dark period with light leads to an inhibition of flowering, while in long-day plants this same switch promotes the process. As we shall see in Chapter 7 the pigment system also controls many other phenomena of plant growth.

Action of the flowering hormone at the shoot tips must also be biochemical, as is the very process of growth itself. Certainly biochemistry is the spirit of modern biology. No other approach has contributed so much in recent years. Thus it is somewhat disappointing to learn that virtually nothing is known with certainty about the biochemistry of the flowering process. We have some ideas, and they will be discussed in Chapter 9, but concrete and specific information still belongs to the future.

5. *Morphogenesis or the Origin of Form*

The final aspect of the flowering process which we will consider is the transformation of the meristems from the vegetative to the reproductive condition. In the cocklebur and perhaps most plants which are sensitive to photoperiod, the flowering hormone is translocated from the leaf to the shoot tips, where it causes this redirection of growth. The change seems to begin essentially at the moment when the hormone arrives, and the subsequent rate of development of the flower buds is proportional to the amount of hormone which reaches the meristems.

It could well be that this aspect of the flowering process has the most fundamental biological significance. When we think of the nearly infinite variety of biological structures, the origin of form takes on considerable interest. Here is the real essence of the relationship between diversity and uniformity in biology. Our observations have convinced us that morphogenesis follows essentially the same pattern in all living things: cells divide, enlarge, and then specialize (differentiate). The secret of diversity in the resulting tissues, organs, and organisms must lie in the differentiation step. During growth the cells are specializing in specific ways that will result in special final organized forms or structures. The degree of coordination of this process is truly fantastic. Only cancerous growth and the occasional monster seem to have escaped this coordination. Since morphology is an inherited trait, all of this coordination and final structure is under control of the genes.

In flowering we have an excellent situation for study of this phenomenon. The shoot tip carries out the intricate steps of morphogenesis which produce stem and leaves, with branches and their shoot tips in the leaf axils. Upon arrival of the flowering hormone all of this changes. The complex flower, with a highly specific form for each kind of plant, is now produced. It appears that the genes which ultimately control the production of leaves and elongated stem are turned off, and the genes for flowers are turned on. Since the flower parts may be thought of as modified leaves, it seems likely that only some of the first set of genes are turned off, but obviously some new ones are turned on. And all of this takes place in response to our chemical substance, the flowering hormone. If morphogenesis in general is a response to chemical substances, study of the flowering process from this standpoint becomes of extremely broad and fundamental significance.

The disappointing thing is that we know little more about the topic than has already been stated above. The problem will be mentioned again in the last chapter along with a summary of what has been done so far to try to solve it, but at this stage very little is known.

The initiation of flowers is a change-over from the indeterminate to the determinate form of growth. The indeterminate form of growth of a plant stem confers potential immortality to the vegetative plant. Leaves, stems and branches can be produced indefinitely, so long as the apical meristem remains alive and active. Thus cuttings might well be taken from the 4000-year-old pine trees in the Sierra Mountains of California, and these might grow for another 4000 years, after which other cuttings could be taken, and so on potentially forever.

The determinate form of growth, typical of most animals, leads to death. The embryo grows, essentially in all directions, until maturity is reached, senescence finally sets in, and death ends the process. Preservation of the species depends upon starting over, so to speak, as single cells from male and female are combined to produce the zygote and new individual.

The flower and subsequent fruit also have the determinate growth form. In a sense, the vegetative meristem is "used up" when it develops into the flower. It is no longer capable of producing the plant body as a whole, but only the determinate flower parts — and of course the gametes which may form the zygote and new individual plant. Thus the initiation of sex organs exchanges the potential of immortality for the possibility of combining germ plasm to produce a new individual. Might we thus conclude that sex leads to death?

The flowering plants have solved the problem in various ways. The true annuals have made the sacrifice. If in nature the environment (or their own internal metabolism) causes them to convert all their buds to flowers during their first year of life, then they only live one year, preserving the species until the next year only in the form of the seed. The cocklebur is an excellent example. It will live for years (potentially forever) in a greenhouse with artificial light where it never is exposed to a dark period exceeding 8½ hr. It can be killed within 2 months, however, by exposing it to a number of long dark