

The Evolutionary Ecology of Plants

Edited by

Jane H. Bock and Yan B. Linhart



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PREFACE

This book presents a broad view of contemporary research in evolutionary plant ecology. Two major themes provide links between the chapters. One is that an evolutionary perspective is necessary and useful at all levels of ecology. The other is that animals are of utmost importance in the ecology and evolution of plants. Ecology is the study of interactions between organisms and their environments, and ecological studies can focus on individuals, populations of organisms of the same species, or biological communities made up of individuals of several to many species. The study of ecological interactions is becoming increasingly refined. Many early analyses concentrated on immediate or short-term responses of individuals or populations to environmental change. These short-term responses were thought to be relevant in an "ecological" or "proximate" time frame. Long-term responses were thought to occur in an "evolutionary" or "ultimate" time frame and were, therefore, the domain of evolutionists and population geneticists. In addition, many ecologists tended to treat both individuals and populations as entities that could be defined by simply assigning them their scientific names. Inter-individual variation was an annoyance whose impact needed to be ignored or minimized. Gradually, the recognition that ecological versus evolutionary time scales and proximate versus ultimate factors were false dichotomies, led biologists such as L.C. Birch and E.B. Ford to seek an integration of ecology with genetics and evolution. This integration is now being carried out by many biologists who are in the process of helping to develop a vigorous discipline of evolutionary ecology.

Most of the contributors to this volume are intellectual descendents of the 19th century naturalists; but unlike our predecessors, we have had the advantage of being trained additionally in genetics and biometry. There is a tendency in some circles to argue that ecology and evolution are such complex fields that only the reductionist approach can be productive. We intend these studies to demonstrate that no one approach will provide all the answers. We need reductionist and holistic studies, in the field and in the laboratory, using short-lived "model" species with simple life styles, and long-lived wild species that are complex and refractory to simplistic experiments.

In **EVOLUTIONARY DYNAMICS**, examples are provided of ecological settings within which evolutionary changes can occur. The settings range from the multi-celled bodies of higher plants,

where plastids have evolved, to broad geographic regions that have helped shape patterns of variation. The characteristics being studied include chromosomes, plastids, flowers, and genetic architecture of populations. In BREEDING SYSTEMS, we describe some of the elegant solutions elaborated by plants when faced with the problem of being sessile, yet needing to move their gametes about in order to reproduce. These solutions become particularly elaborate when animal pollen vectors are not reliably available. In REPRODUCTIVE BIOLOGY, we illustrate the broad spectrum of life history stages which affect plant reproductive success in some fashion. These stages include the complexities of pollen coverings, developmental pathways leading to seed production, and the impact of yearly variation in insect parasites and rainfall conditions upon total seed output.

PLANT-ANIMAL INTERACTIONS is one of this book's recurrent themes. In addition, we address two specific facets of this phenomenon. One has to do with herbivory and its consequences, and the other is a potential solution to the vexing problem of being eaten: attraction by plants of mercenary arthropod bodyguards. Traditionally, an evolutionary perspective in ecology has been especially sought in studies of single-species populations. In the section on EVOLUTION AND ORGANIZATION IN COMMUNITIES, we illustrate how networks of interactions among species, played out against a background of variable physical conditions, can generate predictable evolutionary consequences.

A favorite activity for students of evolution since Darwin's time has been the study of domesticated species to illustrate the power of selection and its complex outcomes. In EVOLUTION IN CULTIVATED PLANTS, we not only pursue this theme by illustrating the interplay between human and natural selection, but we go beyond it to show that the evolution of cultivated plants may involve an interplay among biological, geographic, historical, and political factors. Finally, in the BIOGEOGRAPHY AND CONSERVATION section, we illustrate how knowledge obtained from studies in evolutionary ecology can be used to suggest appropriate strategies for the conservation of biological resources.

All of the senior authors except for Dr. Stebbins have shared the experience of studying with Professor Herbert G. Baker. This has been of considerable importance to all of us. The breadth of subject matter in this volume reflects his catholic tastes and interests as well as his encouragement to follow our particular interests while working under his guidance. To each of us, he has supplied standards to emulate, if not to achieve. These include intellectual curiosity, tolerance for differing viewpoints, a recognition of the need for breadth of training and field

experience, and, at a personal level, patience, wisdom, and kindness.

Most of these papers were presented at a symposium in honor of Herbert G. Baker that was sponsored by the Botanical Society of America and the Ecological Society of America at their joint meeting in August 1987 at Columbus, Ohio. We are grateful to the program committee of the Botanical Society and to Ohio State University for their help in arranging the program. We give special thanks to the many outside reviewers who helped prepare these papers for final publication. Carl Bock, Robert Cruden, Michael Grant, and Meredith Lane deserve particular thanks for their help. Phyllis O'Connell and Carl Bock, along with our Westview editor, Kellie Masterson, helped generously with manuscript preparation. Finally, we thank the contributors for their enthusiasm for this project and for their fine articles.

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INTRODUCTION

HERBERT BAKER: A RENAISSANCE BOTANIST

G. Ledyard Stebbins

In our culture, we often refer to someone with broad cultural background and interests as a "Renaissance Person." With this designation, we recognize a broad cultural interest, that may not have led to preeminent fame and glory in any narrow field of endeavor, but has made the bearer a welcome addition to any cultural group because of his breadth of interest, his enthusiasm for his listeners, both neophytes and old pros alike, to develop a similar attitude. A renaissance person is one who opens the doors to the temple of wisdom and understanding, both for his own pleasure and satisfaction and, more importantly, for that of others. Without a sprinkling of renaissance minds and attitudes, the world of knowledge and silence would be dull indeed.

Can we speak of a renaissance botanist? In a literal sense, no, since botany as a science was unknown during the historical renaissance centuries. Nevertheless, some botanists have genuine characteristics that we associate with the "renaissance" epithet. They have the breadth, knowledge of several fields, and enthusiasm that make them recognized and revered by all who know them well, and they inspire others with their genuine thirst for knowledge and understanding.

In my opinion, Herbert Baker is the very model of a renaissance botanist. He has achieved this distinction in three different ways.

First, a renaissance botanist is not satisfied with achievements in pure science for its own sake. He seeks to spread the good news to those outside of his field. This can be done both by brilliant lecturing and teaching, and by promoting institutions dedicated to building bridges between scientists and the public. Herbert Baker has been outstanding in both of these accomplishments. Even while working on his Ph.D. thesis, he aided his country while it was passing through the crisis of World War II, serving as a volunteer warden in a civil defense post. His first scientific paper was published during this period (1942), under the title "A red coloring matter from the green leaves of spinach beet." His interest in bringing science to public attention

grow during four years (1953-57) as Professor and Lecturer at the University of Ghana. He already realized that tropical botany is a wave of the future, largely because tropical plants, now in grave danger of decimation and extinction, make many major contributions to the well being of all men. After becoming Professor at the University of California, he became a prime mover in the Organization for Tropical Studies. Nevertheless, he was not blinded by the glamor of the rich biota in tropical rain- and cloud-forests. He realized that the study of tropical weeds could at the same time provide a major source of information for understanding mechanisms of evolution, and also increase the efficiency of crop production in tropical climates. One of the most delightful associations that many of us, and I in particular, had with Herbert was at the Symposium on the Genetics of Colonizing Species, held at Asilomar, California, in 1964. When the then President of the International Union of Biological Sciences, C. H. Waddington, decided that a major function of the Union should be to organize symposia dealing with topics of major scientific and economic interest, such as the effect of human activity in spreading many kinds of animals and plants throughout the world, he gave me the task, as General Secretary of the Union, of organizing the symposium. I immediately realized that I would need expert help, and that Herbert Baker was the key person to provide it. The result was a twofold reward: first, one of the liveliest and most stimulating conferences that I have ever attended, and, second, the origin of a volume that has become a minor classic in the field of organismic evolution and human affairs. Since then, Professor Baker has continued to develop the field of colonizing plant species, both tropical and temperate, via public addresses, talks at symposia, journal papers, and a book "Plants and Civilization."

Through all of this activity, he has never forgotten the importance of direct contact with amateur plant lovers. He expressed this interest during his service as Director of the Botanical Garden at the University of California, Berkeley, where he did much to make it both a beauty spot and a center of scientific information about plants. He continues this activity by teaching a course on "Plants and Civilization," and by showing a real interest in a purely amateur organization, the California Native Plant Society.

Another feature of a renaissance botanist is that he does not let the glamor of public service and approval overshadow his attention to the nuts and bolts of his profession: research that is directed toward other botanists and provides them with principles on which to build sounder edifices of basic knowledge. Throughout

his career, Dr. Baker has remained faithful to this mission. During his earliest period of scientific investigation, he made a major contribution by investigating natural populations of wild pinks (*Melandrium* or *Lychnis*) where he demonstrated the importance of hybridization in plant speciation and evolution, and at the same time showed that a particular character, hairiness on leaves, has the function of protecting the plant from attacks of aphids that can transmit lethal diseases. Later during this period, he followed in Darwin's footsteps by investigating floral dimorphism in the genera *Armeria* and *Limonium*, and uncovered that facts that underlie a generalization often termed "Baker's Law." Populations found near the original center of distribution of a genus usually are sexual and predominantly cross fertilized, while those that have migrated the farthest from this center are usually self fertilized or have reverted to asexual reproduction.

His sojourn in Africa inspired Herbert and his co-workers to gather hard data on a topic that was then controversial: the pollination of flowers by bats. Largely because of their carefully recorded observations, bat pollination became a generally recognized phenomenon in many tropical species. During his tenure at the University of California, his growing interest in weeds enabled him to recognize and characterize plants that have "general purpose" genotypes: the same genotype can be successful in a variety of environments. Finally, his research in recent years, in collaboration with his wife Irene, who is an excellent botanist in her own right, has uncovered many highly important facts about the nectar that in many flowers cross pollinated by animals, serves as the major attractant and reward for the vector. These investigations of pollination biology will for years to come stand as a landmark in unravelling the complexities of plant evolution.

Still another admirable trait of a renaissance man, in any field, is a sense of history. Herbert Baker has this appreciation of his forerunners in large measure, as is evident from reading several of his papers.

Finally, the crowning characteristic of a renaissance botanist is his ability to reach for the stars. He has successfully exploited the aphorism of Theodosius Dobzhansky, "Nothing in biology makes sense without evolution." My first acquaintance with Herbert Baker was when he was, at the invitation of Jens Clausen, a postdoctoral fellow of the Division of Plant Biology of the Carnegie Institution of Washington, at Stanford University. There he exploited and sharpened his ability to work with other scientists in a synthetic approach to the basic problems of evolution. His subsequent career has included participation in

various synthetic and cooperative endeavors. Such cooperation is possible only if a scientist recognized that his won competence, however great it may be, is matched by the equal or greater competence of others. Critical respect for the achievements of scientists working in similar or related fields is essential for making real progress in understanding evolution or any other major scientific problem. These are necessary qualities of a renaissance botanist. Herbert Baker has them to a high degree. All of us who are participating in this symposium held in his honor salute him both for his outstanding stature as a scientist, and his ability to remain a productive, sympathetic and cooperative member of the world's scientific community.

EVOLUTIONARY DYNAMICS

The dynamics of the evolutionary process are studied at many levels. No single method, or species, or line of evidence is likely to provide the critical demonstration of the existence of a complex series of events required for evolutionary change to take place. In this section the authors describe evolutionary pathways favoring new structures and functions in plastids, and new flower shapes. They also describe factors affecting genomic architecture. This architecture can be affected at the level of multiplication of whole chromosome sets, i.e. polyploidy, or at the level of ecological variables that determine genetic recombination and genome fluidity.

The origin of plastids is one of plant evolution's intriguing mysteries. The most likely scenario suggests that they are derived from unicellular algae originally "acquired" as endosymbionts by protists. As multicellular plants evolved, their internal environments provided new opportunities for plastids. Whereas most plastids in the more primitive plants retained their photosynthetic abilities, in more recently evolved multi-celled plants, especially angiosperms, plastids have evolved new functions. Jean Whatley has studied plastid structure and function extensively. Her essay describes the role of plastids as accumulation sites of secondary metabolites that are probably involved in activities far removed from photosynthesis, including developmental control, attraction of pollinators, and deterrence of herbivores.

Evolution is most often detectable because of changes in the phenotypic features of organisms. These features have a morphological and developmental history of their own that often is shaped by changes in ecological conditions. Ecological forces that can affect plant growth and structure include interactions with other species such as pollinators, and the uncertainties of temporally variable resources. Factors affecting the shape of individual plant organs have been of interest to many botanists. It is a common observation that, for most plants, relative amounts and predictability of resources will affect both overall plant size and shape and the size and shape of individual vegetative organs. The shape of flowers is commonly perceived as predictable, hence the dependence of systematics upon floral characters. Yet flowers can vary in shape within species, and this variation often has a genetic basis. This provides the ingredients for evolutionary change.

Because competition affects competitors negatively, it is not

surprising that the flowers of closely related, sympatric species often differ from one another in shape and/or color. This results in the attraction of different pollinators and reduces the likelihood of deposition of allospecific pollen. The details of the evolutionary changes associated with the process of adapting to a new set of pollinators are poorly understood. One reason for this may be that many pollinators are insects. They have characteristic sensory systems, physiologies, and behaviors that are very different from ours. In addition, there are important differences among insect groups in these features. Also, many insect-flower interactions have a long geologic history. In contrast, hummingbirds consist of a relatively small number of species with similar physiologies and behaviors. Their search behaviors depend on a vision system more similar to ours than to that of insects. Finally, adaptation to hummingbird pollination is a relatively recent phenomenon. For these reasons, hummingbirds as pollinators have attracted much attention. The flowers they visit tend to have a certain "standardized" tubular shape and are typically red and odorless. In this volume, Stebbins addresses the evolution of flower shape in hummingbird flowers, treating these shapes as striking innovations. He suggests that such innovations, which lead species in new directions, are important, but very rare and difficult to observe. He surveys the flora of California and demonstrates how a suite of characteristics including flower shape, color, and the ability to grow where hummingbirds are abundant reflect selection by hummingbirds as pollinators. He concludes that various components of flower shape play the most important role in the adaptive process. Adaptation to hummingbirds as pollinators has occurred very often in some families, and Stebbins suggests that these families have in their genomes a latent potential for responding in specific directions in response to these pollinators.

Guerrant has undertaken careful studies of floral development to illustrate how evolution acts very specifically at the morphological and developmental levels. In the current essay, he argues for caution in interpreting evolutionary changes in floral shape. He analyzes reduction in flower size in an autogamous species of *Limnanthes*. Such reduction is often seen in autogamous species and is usually interpreted in adaptive terms. Autogamous species, that do not depend on pollinators for fertilization, can produce small, inconspicuous flowers, and shunt the energy resources they save to another function. Guerrant argues that, in *Limnanthes*, both flower size and autogamy are incidental consequences of selection for faster development and earlier maturation.

The study of polyploids has a long and distinguished history among students of plant evolution. These analyses have demonstrated the central role played by polyploidization in species formation. Polyploids can arise as a result of "spontaneous" multiplication of chromosome sets, in which case there can be variation in number of sets within species. Keeler and Kwankin's contribution highlights this fascinating aspect of polyploidy: the presence of intra-specific variation in chromosome numbers. Such variation is quite common in higher plants, but much more common in some taxa than in others. In the grasses of the Great Plains of central North America, it is especially common, and Keeler and Kwankin attempt to explain why. Their answer is complex and suggests that we may be observing a transitional period in the evolutionary history of certain genera in which one chromosomal complement is being replaced by another.

In a detailed comparative study of the genus *Trillium*, Fukuda demonstrates how different ecological and genetic circumstances in North America and Japan have generated very different patterns of variation and species formation. In North America, speciation appears to have been primarily by allopatric differentiation. Patterns of variation in chromosome morphology are also strongly associated with the extent of environmental heterogeneity. In Japan, polyploid formation in the context of hybridization has been much more important than in North America. Differences in geographic distribution, paleogeologic history, and breeding systems have interacted to generate very different patterns of speciation in the two regions.

The genetic organization of plant populations is the product of interactions between genome organization, spatial and temporal distribution of individuals, mating patterns among these individuals, seed dispersal, germination, and a combination of selective and random events, that determine which plants survive to maturity. These interactions have been studied in temperate plants in this context for centuries. The existence of geographic patterns of variability in forest trees was described in pre-Darwinian times, and was cited by Darwin as evidence for evolution. With increasing concern about the loss of tropical forests, serious efforts are being made to document genetic organization in tropical plants. Hamrick and Loveless make a significant contribution in their essay. They demonstrate that tropical trees are at least as genetically variable as temperate trees, and that trees in general tend to be more genetically variable than other life forms. This tendency for woody plants to be highly variable has been noted on a number of occasions. Linhart's essay on community interactions provides one possible

explanation for this observed pattern. Long-lived woody plants are exposed to extremely variable diversifying selection because of their interactions with large numbers of species of herbivores, parasites and diseases, each of which may generate its own patterns of selection on a plant host. A second finding of Hamrick and Loveless is that the organization of genetic variability can be predicted to some extent by patterns of pollen and seed dispersal: amount of gene flow, usually deduced from pollinators' and seed dispersers' flight capabilities, is correlated with the extent of inter-population genetic differentiation. This finding has been anticipated at the theoretical level but documenting it in a heterogeneous tropical forest is very important.

PLASTIDS IN A CHANGING ENVIRONMENT

Jean M. Whatley

INTRODUCTION

My current research on ultrastructural aspects of plastid evolution and development is far removed from the ecological research that I carried out under the guidance of Dr. Herbert Baker. Nevertheless, I have found over many years that ideas acquired during my ecology days can often provide an unexpected insight or slant to the questions that arise with respect to ultrastructure. The supposed endosymbiotic origin of plastids makes it particularly appropriate to consider the cell as a habitat. Successive stages of plastid development can be regarded as a parallel to successive stages of colonization; productivity (rate of photosynthesis) increases to a peak and then declines during the life of a chloroplast, just as it does during the life of an ecosystem (Kirt and Tilney-Bassett, 1978). The various symbiotic and commensal interrelationships among organisms in an ecological setting can give insight into subjects as different as the control of plastid populations on the one hand, or plastid structure and function in relation to pollination on the other. The forms or functions of the plastids in plant cells at a particular site or at a particular time can have important ecological consequences by contributing, for example to the synthesis of plant protectants as well as attractants or, indirectly, by influencing photoperiodic controls. It therefore seems appropriate here to review some of the ways in which plastids may have evolved in response to a changing land environment.

ENDOSYMBIOSIS

Towards the end of the last century, Schimper (1883) suggested that chloroplasts might have evolved from photosynthetic organisms that were once free-living, but were later taken up by unicellular hosts whose endosymbionts they became. Following the discovery by Ris and Plaut (1962) that chloroplasts had their own (prokaryotic-type) DNA, interest in the Schimper hypothesis revived and stimulated many ultrastructural and biochemical research projects and symposia. As a result of these, the original hypothesis has been somewhat modified but it has now become widely accepted that chloroplasts had an endosymbiotic origin, though many more detailed aspects of the general evolutionary scheme still have to be resolved. Briefly, it is now thought that the chloroplasts of red

(rhodophyte) and green (chlorophyte) algae and land plants may have evolved from prokaryotic algal symbionts. By contrast, the more complex chloroplasts of the remaining algal phyla (Chromophyta sensu Christensen [1962] and Euglenophyta) may have evolved from a range of eukaryotic algal symbionts in which prokaryotic-type chloroplasts were already established. The similarity in pigmentation, ultrastructure, and sequencing data clearly point to a cyanobacterium (blue-green alga) as the ancestor of the red algal chloroplast. There is some difference of opinion as to whether the ancestral "green" chloroplast was a cyanobacterium with chlorophyll a and phycobilins as its photosynthetic pigments or another prokaryotic algal that already had both chlorophylls a and b, but lacked phycobilins 1/M the ancestor of the modern *Prochloron* (Dodson, 1979; Gibbs, 1981; Whatley and Whatley, 1981; Stackenbrandt, 1983; Cavalier-Smith, 1982).

THE CELL AS A HABITAT

The establishment of an alga as an endosymbiont would immediately provide the host with a new and continuing source of food through algal photosynthesis, and the alga, having avoided being digested in a phagocytic vacuole, would have found a new, relatively stable habitat, free of other predators, where nutrients could be intercepted from some of the host "waste" products. Some metabolites produced in excess of immediate requirements could be stored temporarily in the plastid or in the cytoplasm. Others of no potential use would be concentrated in vacuoles or eliminated directly from the cell, and, as Church (1919) suggested, some of these would have contributed to newly evolving external structures such as scales and cell walls. In modern corals, for example, up to 60% of the total carbon fixed by algal symbionts is exported as mucolipid and mucopolysaccharide (Crossland et al., 1980).

Taylor (1983) has suggested that, for permanent endosymbionts, continued isolation from their former gene pool would have been likely to lead to divergent genetic change similar to that found among small island communities. The evolution of species-specific strains among modern symbionts seem to reflect such a change. Through the transformation of an algal endosymbiont into a chloroplast must also have been accompanied by considerable physiological and structural modification, the chloroplast, once established, seems to have been a highly conservative organelle. The number of new structural forms that have evolved is small. An important part of the integration of the

chloroplast into the cell life cycle has been the loss by the plastid of much of its own DNA; this has surely been a major factor in the apparent structural stability of the chloroplast. Although plastids retain the capacity to code for some of their own polypeptides, it is the cell nucleus which exerts overall control. As the majority of plastid modifications are limited to the angiosperms, it seems that the nucleus has been very slow to influence plastid forms.

Most green algae and some lower land plants have a single, large, most commonly cup-shaped chloroplast in each cell. However, there may be a major and ancient distinction between those green algal species whose chloroplasts have irregularly stacked thylakoids (the prokaryotic *Prochloron* and members of the Ulvophyceae and Chlorophyceae, as classified by Steward and Mattox in 1984) and those which have grana (members of the Charophyceae including the Zygnematales and Coleochaetales); it is from the latter that land plants are believed to have evolved (Figs. 1, 2, and 3). Modifications in the structure of plastids seem to have been mainly influenced by two major trends; (1) the evolution of multicellular algae with the appearance of meristems and with the potential for cell specialization; (2) colonization of the land and the evolution of a wide range of adaptive structures which assisted survival under terrestrial conditions.

MULTICELLULAR ALGAE AND MERISTEMS

In endosymbiotic partnerships, the symbionts are liable to be eliminated by the host under conditions of stress (Cook, 1983). By contrast, plastids very seldom suffer "predation", though those from one parent are often eliminated during sexual reproduction (Whatley, 1982). Indeed it is very difficult to eliminate plastids even from organisms like euglenoids (Kivic and Vesik, 1974), which can feed themselves heterotrophically. Instead when nutrients, particularly nitrogen, are in short supply or when growing conditions are otherwise unfavorable, the plastids tend to dispense with their thylakoids, a response which may save energy. In unicellular algae which had come to depend on their chloroplasts as their sole source of nutrition, any loss of photosynthetic activity must of necessity have been of only short duration or would have required a major reduction in other metabolic activity as, for example, during dormancy. Plastid dedifferentiation may well have arisen in the first place as a response by the cell to stress conditions irregularly imposed by the unpredictable environment.

Plastid dedifferentiation as a regular phenomenon within the algal life cycle perhaps first appeared in the male gamete (plastid degeneration) or in zygotes (becoming dormant or possibly

		Chloro-plasts				Pro-plasts		Chromo-plasts			
		1	2	3	4	5	6	7	8	9	10
Angiosperms	Monocots										
	Dicots										
Gymnosperms	Gnetales										
	Coniferales										
	Ginkgoales										
	Cycadales										
Ferns	Leptosporangiate										
	Eusporangiate										
Lower vascular plants	Equisetum										
	Isoetes										
	Lycopodium										
	Selaginella										
	Psilotum										
	Anthocerotae										
Non-vascular plants	Hepaticae										
	Musci										
	Charophyceae										
Charophyceae	Charales										
	Coleochaetales										
	Zygnematales										
Ulvophyceae											
Chlorophyceae											

- 1. Cup-shaped chloroplast (or plate)
- 2. Discoid chloroplast
- 3. Grana present
- 4. Pyrenoid present
- 5. Non-green plastids in reproductive cells and meristems
- 6. Secretory non-green plastids
- 7. Eyespots
- 8. Chloro-chromoplasts
- 9. Globulous chromoplasts
- 10. Other chromoplasts

Figure 1. Some modifications in plastid structure.

undergoing meiosis) and must have required the evolution of new internal control mechanisms. The introduction of proplastids to the egg cells, young embryos and meristems, first, of the sporophyte and, later, to some specialized cells of the gametophyte generation may represent several successive evolutionary steps. The fragmentary information presently available suggests that today chloroplasts rather than proplastids are found in the gametophytic meristems of hornworts, liverworts, mosses, and ferns though proplastids are characteristic of some sporophytic meristems (Duckett and Renzaglia, 1988). Proplastids are also characteristic of meristems in some red and brown algae. Thus the evolution of proplastids is not a modification unique to the ancestors of land plants. Nevertheless, the proplastid may well have been the original key to the development of new and functionally more flexible forms of plastid in specialized cells which represent adaptations to the land environment (Fig. 1). Certainly as land plants have evolved, the proportion of the plant body given over to photosynthesis has conspicuously declined and most of the new plastid forms found today in land plants lack chlorophyll.

PLASTID DEVELOPMENT

Before considering the evolutionary responses by plastids to colonization of the land, it is helpful to describe the basic pattern of plastid development as it is found today in the photosynthetic tissues of angiosperms. With this as background it is easier to describe the various "new" forms of plastic that seem to represent adaptations to the changing environment.

The chloroplast is only one of several different types of plastid found in angiosperms. Some of the less familiar forms are found only as stages during development; others are mature forms with a function which is non-photosynthetic. Assuming that plastids evolved from photosynthetic endosymbionts, then the photosynthetically active chloroplast is undoubtedly the most ancient plastid form. From an historical point of view, the non-photosynthetic forms of plastid, including the proplastids, should therefore be considered as derived. However, for practical purposes, it is usually convenient to consider plastid development, particularly in angiosperms, as beginning with the proplastid stages present in the ripe seed and proceeding to the chloroplast stages of mature leaves or other green organs. The pattern of plastid development from the simplest proplastid stages to the various mature (climax) and, finally, senescent types can be followed both in time, during synchronous development, and in space, from a basal meristem upwards along a file of cells, in a

Figure 2. Part of a filament of the green alga, *Enteromorpha* sp. (Ulvophyceae) showing the large cup-shaped chloroplast with its irregularly stacked thylakoids and pyrenoid (P).

Figure 3. A typical chloroplast from *Fittonia verschaffeltii* showing the grana in both longitudinal and transverse section.

Figure 4. Part of the endosperm mother cell of *Phaseolus vulgaris* showing the extensive development of endoplasmic reticulum around the amyloplasts.

Figure 5. Globulous chromoplasts in the upper epidermis of a petal of *Caltha palustris*.

Figure 6. Sieve element plastid from the fern, *Matteucia struthiopteris*, showing osmiophilic material within a fragment of the thylakoid sac.

Figure 7. Sieve element plastid from the fern, *Salvina* sp., showing proteinaceous crystals in the depleted stroma.

Figure 8. Sieve element plastid from the angiosperm, *Phaseolus vulgaris*, showing the specialized starch in the depleted stroma.

Figure 9. Electron dense material within the thylakoid sac and within a membrane-bound-body of a developing chloroplast of *Impatiens* sp.

Fig. 7, scale bar = 0.5 μ m; Figs. 2-6, 8 & 9 = 1.0 μ m

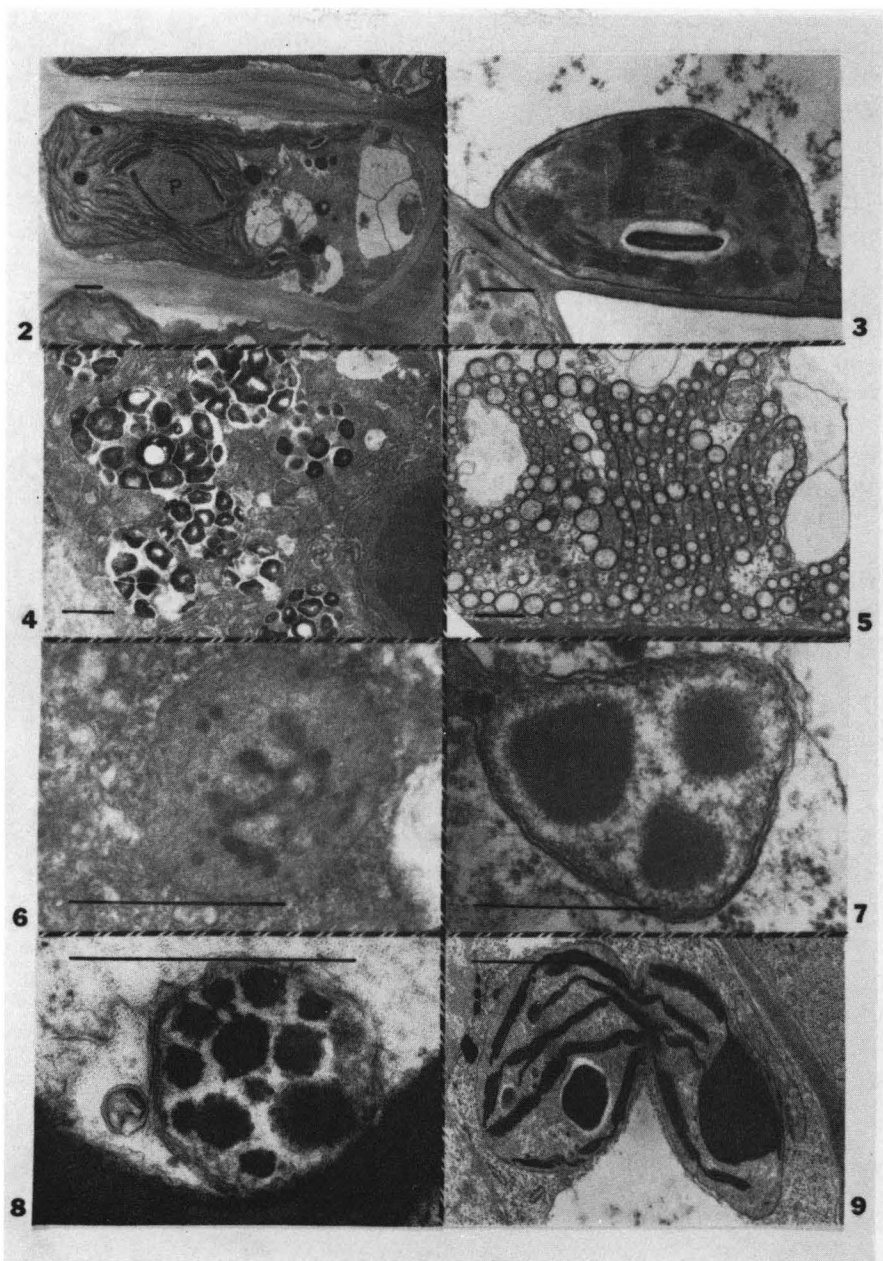


Figure 10. A crystalline membrane-bound body in a young chloroplast of *Spinacia oleracea*.

Figure 11. A chloroplast of *Equisetum telmateia* showing osmiophilic material in the plastid envelope and in the associated endoplasmic reticulum.

Figure 12. A chloroplast from the tern, *Pilularia globulifera*. There are no obvious modifications in plastid structure, but note the osmiophilic deposits in the developing vacuoles (→).

Figure 13. Chromoplast segments in yellow flowers of *Narcissus pseudonarcissus*, showing the concentric membranes (→) of one and the accumulation of strongly electron dense material in the envelope sac of another.

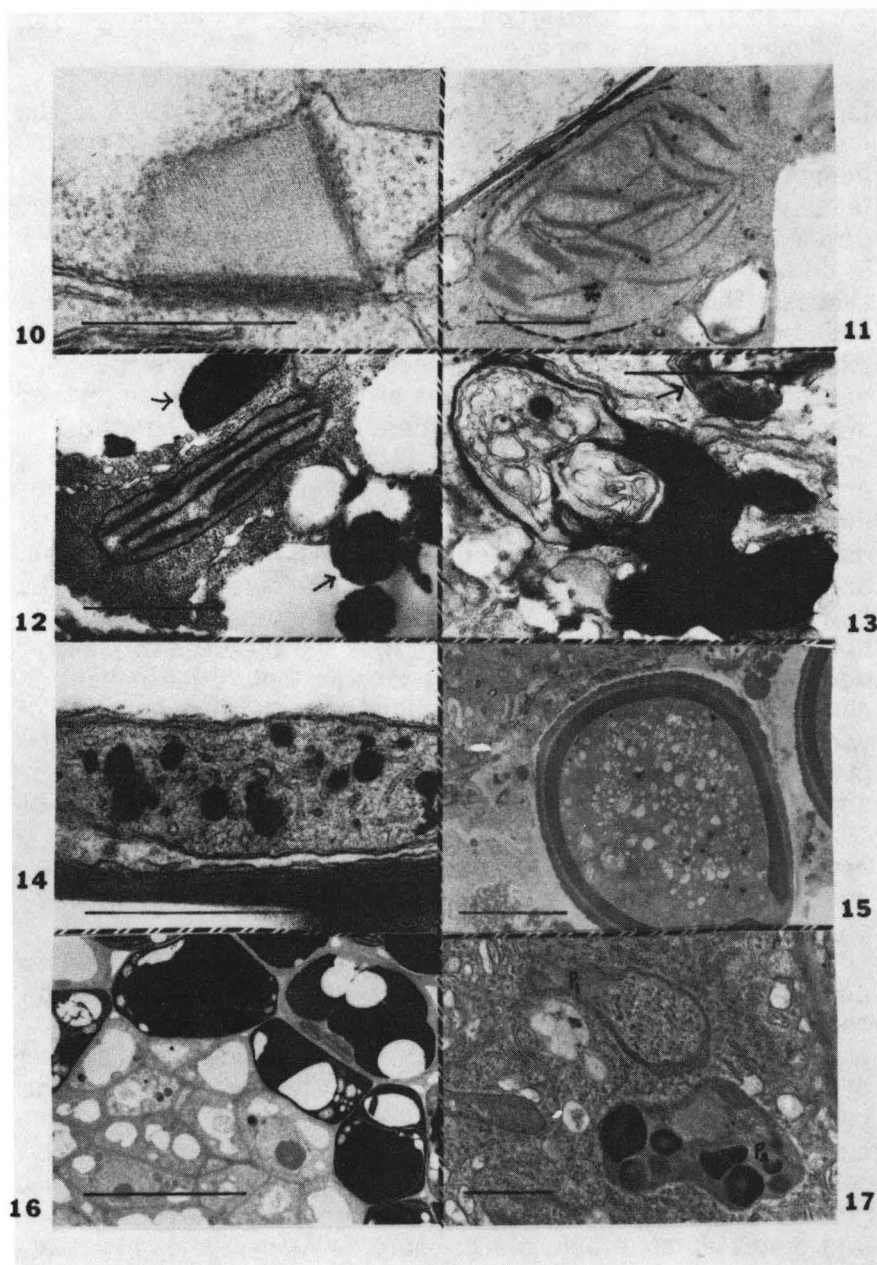
Figure 14. Part of a plastid in a white petal of *Phaseolus vulgaris*. Note the osmiophilic globules and the tubular complex in the stroma.

Figure 15. Degraded tapetal cells and pollen grains in a pollen chamber of *Phaseolus vulgaris*.

Figure 16. Secretory and non-secretory cells in a leaf of *Theobroma cacao*. In the secretory cells, the osmiophilic material lies in the cytoplasm, not in the vacuoles.

Figure 17. Pleomorphic plastids (P) with electron dense stroma, in the mucilage-secreting cells at the periphery of the root cap of *Phaseolus vulgaris*.

Figs. 15-16, scale bar = 10.0 μm ; Fig. 10 = 0.5 μm ; Figs. 11-14 & 17 = 1.0 μm .



way that is analogous to following the successional stages of plant communities, either at various time intervals from the onset of colonization to the climax community, or along a transect of, say, sand dunes of increasing age.

Five basic stages have been identified as leading to the formation of chloroplasts in angiosperms (Whatley, 1977). The first stage is the simplest form of proplastid, essentially a double membrane-bound sac containing stroma and a fragment (a template?) of thylakoid ribbon (Whatley, 1983a). As development begins, the plastid starts to accumulate starch which is derived from reserves mobilized from older photosynthetic tissues. This amyloplast phase is usually brief and the plastid soon begins to change in shape from spheroidal to pleomorphic. At this third stage, invaginations of the inner plastid envelope become common and there is some slight extension of the thylakoid system. During the period when starch is accumulating, and probably also when it is being lost, the plastid is temporarily ensheathed by rough endoplasmic reticulum (Fig. 4). Following the pleomorphic third stage, the plastid assumes its mature discoid shape. There is marked extension of the thylakoid system and the number of incipient grana or bithylakoids increases. The formation of true grana leads to the final stage of development, that of the mature chloroplast (Fig. 3). The progress of plastid development can be interrupted or diverted before the mature chloroplast stage is reached and so lead to the development of variant forms. It is by such diversion that distinctive forms of plastid (e.g., etioplasts or chromoplasts) develop. The inhibition and reestablishment of the normal pattern of development may be caused by factors that are either external, reflecting local environmental conditions (etioplasts formed in darkness) or internal, reflecting the overall nuclear control (most chromoplasts).

In annual plants or in deciduous species, the chloroplasts and their thylakoid systems continue to increase in size for some time prior to the onset of senescence, at which time the photosynthetic apparatus becomes degraded and increased amounts of carotenoids are synthesized. In species in which the leaves or other photosynthetic organs persist for several years, the chloroplasts sometimes undergo dedifferentiation during a regular cold or dry season, followed by redifferentiation when active growth is again possible, i.e. the chloroplasts revert temporarily to a proplastid state before once more developing into chloroplasts (Gaff, Zee and O'Brien, 1976; Senser, Schötz and Beck, 1975; Whatley, 1978). Thus chloroplast development is not an unidirectional system which invariably ends with senescence but

rather a cyclical phenomenon which is, perhaps, best represented as a series of developmental waves. The onset of senescence and divergence to variant forms are not restricted to the chloroplast stage but can take place at any point within the cycle. Although it was suggested above that ripe seed was a convenient starting point for developmental studies, it should be recognized that this "starting point" is merely the trough of a wave in which the plastids may have already undergone various degrees of differentiation during seed growth and of dedifferentiation during seed dehydration and ripening while still on the parent plant. From the point of view of the evolution of new plastid forms and functions, the most important aspect of the plastid developmental cycle may well be the capacity to dedifferentiate and redifferentiate.

Colonization of the Land. As land plants have evolved, the proportion of tissues devoted to photosynthesis has declined. Thus, within each plant, an increasing proportion of the cells and their plastids have become available for other metabolic activities. For what functions have the plastids become specialized and when did these modifications take place?

It has long been recognized that the evolution of land plants has involved the development of new structures which could provide, inter alia, a means of anchorage and of taking up nutrients and water from the soil, a vascular system and mechanisms for waterproofing, structural strengthening, and exchanging gases with the atmosphere. Essentially, land plants had to fulfill these requirements by using and modifying the components and biochemical pathways already available to their green algal ancestors. And the process of modification has had to continue to meet the changing conditions of the land habitat to which the evolving plants and accompanying animals have themselves contributed. It might be thought that a survey of the plant species extant today might reveal a sharp break between the plastids in green algae and those in land plants. This is certainly not so. Characteristically algal features like cup-shaped plastids (Figs. 1 and 2) are retained by some land plants; "new" forms of plastid, like chromoplasts, can be identified in the alga *Chara* (Pickett-Heaps, 1975; Whatley, 1983b). Among modern land plants, the great majority of the known modified plastid forms are restricted to the angiosperms, but we do not know if similar modified plastids were present in lower plants which are now extinct or if their evolution has gone hand in hand with evolution of the angiosperms and perhaps even, indirectly, of the insects and other animals. The overall control by the nucleus of most

chloroplast biosynthesis would presumably allow chloroplast metabolism to be diverted to activities in keeping with those required by the plant as a whole.

In addition to their main photosynthetic activity, green algal chloroplasts have two important abilities; (1) to accumulate in their stroma excess photosynthetic reserves like starch, as well as pyrenoid protein and globules of lipids and carotenoids and (2) to exchange metabolites with the cell cytoplasm. The evolution of new forms of non-green plastid has apparently been accompanied by the exploitation of these two abilities. As land plants have become increasingly complex, some algal features have been lost (*e.g.* the capacity to form pyrenoids), but in general, there seem to have been (1) changes in the use to which established metabolites are put; (2) a noticeable increase in the range of metabolites, particularly of secondary plant products, to which the plastids contribute; (3) an increase in the number of sites within the plastid where reserves of these metabolites can accumulate; (4) an increase in area of the surface across which the plastid may exchange metabolites with other cell compartments.

The Changing Use of Metabolites

Plant geotropism is believed to be mediated by sedimenting particles or statoliths. In rhizoids of the green alga, *Chara*, the gravity-perceiving particles are crystals of barium sulfate; in land plants the statoliths are amyloplasts. The initial function of the amyoplast was presumably solely that of stage of photosynthetic reserves (Fig. 4). Sedimenting amyloplasts that are associated with a geotropic response are found in localized groups of cells in a range of different organs, and in taxa as diverse as bryophytes and angiosperms - in the stems of mosses, in the setae of moss and liverwort sporophytes, and perhaps also in the underground axes of some liverwort gametophytes (Osborne, pers. comm.; Duckett and Renzaglia, 1988), in the root cortex of *Selaginella* and in the root caps of *Equisetum*, *Isoetes*, ferns and gymnosperms. In different species of angiosperms, geoperceptive amyloplasts can be found in the root cap, the root cortex, the stem endodermis and in leaf nodes (Osborne and Wright, 1977; Whatley, 1983a). Nor is gravity perception the only specialized function of amyloplasts. In petals of some members of the Ranunculaceae, starch is only accumulated in the plastids of the subepidermal layer. As these cells mature most of their contents disappear, but the numerous large starch grains remain and apparently serve as attractants for pollinating insects by causing light to be reflected back from the

petal surface (Kay, Daoud, and Stirton, 1981; Whatley, 1984; Brett and Sommerard, 1986).

Although phycobilins and the chlorophylls are the main photosynthetic pigments, carotenoids may also participate in the process, both directly in light absorption and indirectly, by preventing photooxidation. These carotenoids are localized in the chloroplast membranes. In many algae which are motile, or have motile stages in their life cycle (gametes or zoospores), additional carotenoids are assembled in localized arrays of globules, the so-called eyespot, at a well-defined site often close to a flagellum, either within the cell's single plastid or within one only of its several plastids. It has frequently been assumed that the eyespot plays a role in flagellar movement, possibly as a photoreceptor or, more possibly, by shading the flagellar base, but its function, if any, remains unclear. Although eyespots are characteristic of many green algae like *Chlamydomonas*, they are never found in those taxa which are thought to be ancestral to land plants. However, during sexual reproduction in *Chara*, carotenoid globules accumulate in all the chloroplasts in each cell within a single layer of cells of the antheridial jacket. Thus, *Chara* can produce plastids which have characteristics of both chloroplasts and chromoplasts. This form of chloro-chromoplast can also be found in land plant taxa ranging from bryophytes to angiosperms (Fig. 1), usually in ancillary reproductive tissues (Duckett, 1975; Pickett-Heaps, 1975; Whatley, 1985). Though carotenoids in the chromoplasts of *Chara* may retain a protective role those in chromoplasts of most land plants (Figs. 5 and 13) have assumed the very different function of attracting spore, seed, and pollen dispersing animals.

The Introduction of New Metabolites

Plastids synthesize or contribute to the synthesis of many metabolites, including carbohydrates, amino acids and some proteins, photosynthetic pigments, lipids, and isoprenoids. Many of these metabolites undergo further modification within other cytoplasmic organelles and some are later excreted from the cell. In algae the excreted products can be used in several ways. Polysaccharides can be incorporated into organic scales, cellulose fibrils, and mucilages, for example, and deposited as a protective covering immediately outside the plasma membrane; mucilage can be used to aid movement as well as to provide a physical barrier against predation. An extruded glycoprotein helps to promote sexual reproduction in *Chlamydomonas*; phenolic and various compounds can act as protectants against predators, as growth promoters or as growth inhibitors (Hellebust, 1974).

Some of the metabolites excreted by the cells of land plants are similar to, but others are very different from, and more varied than, those released by the cells of green algae; the latter clearly reflect the changing requirements imposed by dynamic terrestrial ecosystems. Nevertheless, the general functions of land plant plastids remain essentially similar to those of algal plastids. Mucilages are produced by many land plants, including bryophytes; they may provide a useful antidesiccant. Some of those released by vascular plants can still assist movement, but by lubricating roots. Polysaccharides continue to be incorporated into wall celluloses, but many of the walls are strengthened by the inclusion of lignin and exterior walls of aerial cells are coated by waxes and cutins which reduce water loss. The evolution of insects and other herbivores has been accompanied by the evolution of new and increasingly different plant protectants; the biochemical diversity of inhibitory, stimulatory, attractant, and recognition compounds has also increased.

The evolution of land plants has been accompanied by a conspicuous increase in the range and complexity of secondary plant metabolites, and the plastids have been implicated in the synthesis of many of these or of their associated carbohydrates (Jensen, 1985). Indeed, Kirk and Tilney-Bassett (1978) raise the question of whether the plastids in higher plants might not be the sites of synthesis of all terpenoid secondary products and, perhaps, even of virtually all phenolic secondary products. As these authors point out, such a capacity would imply for the plastids an "extraordinary range of biosynthetic capabilities."

Among the secondary products to which plastids may contribute are the flavonoids (Jensen, 1985). These are perhaps best known in the forms of anthocyanins and flavonols, pigments which normally accumulate in the vacuoles, and of lignin. The flavonoids function as attractants or insect guides in many flower petals, usually being concentrated in cells of the upper epidermis (Kay et al., 1981). Swain (1975), for example, has reviewed the main aspects of flavonoid evolution in relation to the evolution of green plants, starting with the appearance of glycoflavones in the algae, *Chara* and *Nitella*, continuing with the later diverging biochemical steps which may have led to the introduction on the one hand of the lignins and on the other of the pro-anthocyanidins (colorless to man) in lower land plants and leading to the complex flavonoids in advanced angiosperms.

New Sites of Forms of Metabolite Accumulation

The Stroma. The metabolites accumulated by the plastids of green algae all appear to be deposited in the stroma. Though the

capacity to form pyrenoids has been lost by most land plants, starch and globules of lipid and carotenoid continue to be laid down, but sometimes in modified form. In the Mullerian bodies (specialized storage organs) of *Cecropia peltata*, for example, glycogen accumulates in the plastid stroma instead of starch; this glycogen provides food for "guardian" ants. In cells elsewhere in the plant, starch is formed in the usual way (Rickson, 1976). In epidermal cells of some members of the Liliaceae, Amaryllidaceae and Orchidaceae and in the cacti *Echinocereus* and *Echinocactus* photosynthetic reserves are stored in large oil droplets (summarized in Kirk and Tilney-Bassett, 1978).

Chloro-chromoplasts belong to the simplest class of chromoplast; they are found in some algae and in many lower land plants. During evolution on the land, the range of carotenoids synthesized has increased enormously (Goodwin, 1980) and the structure of chromoplasts has become more varied. Sitte (1974) has identified five additional classes of chromoplast. Of these the globulous chromoplasts are the most common (Figs. 1 and 5), being found in the gymnosperms, *Taxus baccata* and *Ephedra aphylla* (personal observation; specimens of *Ephedra*, courtesy of Prof. A. Fahn of the Hebrew University, Jerusalem) and in many angiosperms. They resemble chloro-chromoplasts in that the carotenoids are concentrated in globules in the stroma, but they lack thylakoids. In other classes of chromoplast, the carotenoids are assembled as fibrils or tubules in the stroma, within the sace of other tubules, in proliferating envelope membranes or as crystals within thylakoid sace. The ripe red seeds of the cycads, *Cycas revoluta*, *Macrozamia lucida* and *Zamia purpurea* are the only sites in non-angiosperm species where chromoplasts other than the two globulous classes have so far been found (Whatley, 1985).

In young chloroplasts, the stroma also appears to be the site of accumulation of some thylakoid and other precursors materials either produced in excess of immediate requirements or held temporarily in a suitably accessible form when the normal developmental processes become imbalanced. Stromacenters, possibly comprising large subunits of ribulose-bis-phosphate carboxylase, may form in the plastid stroma under conditions of stress (Kirk and Tilney-Bassett, 1978; Wellburn, 1982). Usually large clusters of phytoferritin particles have been observed in the stroma in plants suffering from iron deficiency (Platt-Aloia, Thomson and Terry, 1983) and in the hypocotyls of bean seedlings grown in darkness (Whatley, 1977).

In many cells engaged in the secretion and/or transport of specialized and often secondary products, the unusually high

electron density of the stroma points to the accumulation there of other as yet undetermined metabolites. Many ultrastructural investigations of secretory tissues (reviewed in Fahn, 1979 and in Werker and Zamski, 1985) concentrate on the endoplasmic reticulum and the Golgi apparatus. Few of the published micrographs show plastids at a sufficiently high magnification to allow a detailed analysis of their structure. Thus, the range of secretory tissues for which adequate information about the plastids is available is unfortunately restricted.

Intra-membrane Sacs. There are two different types of intra-membrane space which have been exploited by land plants as sites of metabolite accumulation, (1) between the two membranes of the plastid envelope and (2) the intrathylakoid space. The extent to which these two sites are structurally contiguous remains uncertain. It is, however, probable that continuity is more frequent between the envelope space and the apparently envelope-derived, non-photosynthetic, concentric lamellae characteristic of some angiosperm chromoplasts, than it is between the envelope space and "true" photosynthetic thylakoids (Thomson and Whatley, 1980; Liedvogel and Falk, 1980; Spurr and Harris, 1968). Even in the absence of structural continuity, material may be moved intermittently between the envelope and the thylakoid spaces by way of vesicles and tubules (Figs. 13 and 14). Thus, channels may be made available for the movement of metabolites. The intramembrane compartments of the plastid also have the potential to provide a large space for storage, and an extensive surface area for contact with the plastid stroma.

The sacs of both the plastid envelope and the thylakoids have been stated to be the site of accumulation of essential oils, the volatile attractants produced, inter alia, in the "sprouting" glands of the angiosperm, *Dictamnus albus* (Amelunxen and Arbeiter, 1967). The fruits of *Phellodendron amurense* also have plastids that are believed to be the site of biosynthesis of the essential oil (Heinrich and Schultze, 1985). In young glands, the site of accumulation is thought to be in tubuli within the stroma, but in older glands the osmiophilic deposits lie in the space between the membranes of the plastid envelope. In plastids in the digestive glands of the carnivorous plant, *Dionaea muscipular* Ellis (Robins, 1978), the intramembrane spaces accumulate an electron-lucent material of unknown composition.

The intramembrane space of the plastid envelope seems to be even more effectively exploited in the petals of some white and also pigmented cultivars of *Narcissus* (Whatley and Whatley, 1987). The orange and yellow flowers of *Narcissus* species

contain chromoplasts in which the carotenoids are concentrated in envelope membranes which have proliferated to form several concentric rings. In spite of the absence of carotenoids, the structure of the plastids in white floral tissues of some cultivars is indistinguishable from the of the membranous chromoplasts of the pigmented flowers. In both the pigmented and the nonpigmented cultivars the envelope sac becomes greatly dilated and filled with a strongly electron-dense deposit as the petals age (Fig. 13). The product stored may well be an essential oil and the volume must be considerable. It has been suggested that such osmiophilic deposits in secretory cells may be artifacts of fixation (Galatis, Katsoras and Apostalakis, 1978). However in leaves of *Equisetum telmateia* (Fig. 11), dilations of the plastid envelope which are associated with osmiophilic deposits following chemical fixation are also present when the tissue is subjected to rapid-freezing and freeze-fracturing (McLean, Whatley and Juniper, 1988).

Though the intramembrane spaces of the thylakoids and the plastid envelope may both be used for the accumulation of some metabolites, the accumulation of others appears to be restricted to sacs provided by the thylakoids, *sensu stricto*. Today, the simplest plants which use intra-thylakoid spaces in this way are apparently the hornworts and mosses, but similar deposits are also found in some lower vascular plants (Fig. 6). The stored material is of moderate electron density, sometimes amorphous, sometimes granular, sometimes crystalline, but of unknown composition. In lower land plants, the cells containing these modified plastids are all concerned with conduction - transfer cells in gametophytic tissues of some hornworts (Duckett and Renzaglia, 1988), the leptoids of the moss, *Polytrichum*, and the sieve elements in some lower vascular plants (e.g., Burr and Evert, 1973; Hebant, 1974; Dute and Evert, 1977; Warmbrodt and Evert, 1979a and 1979b; personal observation). In other ferns, in gymnosperms and in angiosperms, sieve element starch and/or protein are stored as granules, crystals or fibrils, but these are in the stroma not in thylakoid sacs (Fig. 7 and 8). The modifications in plastid structure in conducting tissues of some bryophytes and of lower vascular plants may have been among the first, and hence the longest-established to be specifically associated with particular types of cell in land plants. It is therefore interesting that the sieve elements of gymnosperms and angiosperms have the only plastids in which structural modification has been successfully correlated with taxonomy (Behnke, 1972). The presence of modified plastids in so many different types of cell engaged in the secretion or transport of

specialized metabolites may be a further reflection of the early evolution of new forms of plastid into conductive tissues.

The thylakoid sac can also be used for the accumulation of other metabolites (Figs. 9 and 10). In algae, the ribulose-bis-phosphate carboxylase, which seems to be the main constituent of pyrenoids (Holdsworth, 1971), lies in the plastid stroma (Fig. 2); the deposit is almost always granular. As described above, this protein may also be deposited in the stroma of plastids of sieve elements in some species of land plant, or as stromacenters, under conditions of stress. However, in mesophyll chloroplasts of spinach (Fig. 10), ribulose-bis-phosphate carboxylase has been identified as the main constituent of initially granular but subsequently crystalline deposits, lying not in the stroma but within the thylakoid sac, i.e. in the so-called membrane-bound bodies (Sprey and Lambert, 1977; Platt-Aloia and Thomson, 1977). Proteinaceous membrane-bound bodies have been observed in the plastids of many angiosperms and in some lower plant groups. However, cytochemical analyses of their contents in plastids of a few of the angiosperm species have suggested the probable presence of peroxidase and polyphenol oxidase rather than of ribulose-bis-phosphate carboxylase. Thus, the intrathylakoidal contents of the membrane-bound bodies may vary (Henry, 1975a, b; Hurkman and Kennedy, 1977; Lazorovits and Singh, 1986). It has already been mentioned that in some angiosperm chromoplasts, the carotenoids form crystals which are initiated within the thylakoid sac (Harris and Spurr, 1969). May this intrathylakoid site provide, for these carotenoids and for some proteins, a protected surface which is particularly suitable for the crystallization process?

Increase in Transport of Exchange Capacity

Some substances accumulated in plastids are only released when the cells and their plastids break down. These include essential oils, carotenoids and the precursors of pollen sporopollenin (Fig. 15), which originate in the tapetum (Stanley and Linskens, 1974). Other substances including sugars and components of phenolics, are regularly released through the plastid envelope.

Association with Endoplasmic Reticulum. The endoplasmic reticulum and Golgi apparatus are important in the further metabolism and transport across the cell of sugars, proteins and other metabolites originating in the plastids. In many bryophytes and lower vascular plants, the plastids are surrounded by a sheath of endoplasmic reticulum; occasionally the

plastid envelope can be seen to be contiguous not only with the endoplasmic reticulum, but also with the envelopes of other organelles (Crotty and Ledbetter, 1973; Whatley, 1977; Duckett and Renzaglia, 1988). However, recent work on rapidly-frozen, freeze-fractured tissues suggest that the endoplasmic reticulum and the plastid envelope may become contiguous much more frequently than had been indicated by chemical fixation techniques (McLean, Whatley and Juniper, 1988). The establishment of such intra-membrane channels would certainly promote the exchange of metabolites between the two organelles. In some ferns and in *Equisetum* (Fig. 11), strongly electron dense deposits (possibly tannins of phenolic compounds) accumulate within the plastid envelope, in the endoplasmic reticulum and often, also, in the vacuoles. In the other ferns there is no obvious association between plastids and endoplasmic reticulum, although phenolic compounds are accumulated in the vacuoles of many species (Fig. 12). An association between plastids and endoplasmic reticulum has been reported in a few green algae (Gibbs, 1981) and in many lower land plants but in gymnosperms only in *Ginkgo biloba*, *Juniperus communis* L. and the resin canal cells of *Pinus pinea* (Stewart and Gifford, 1967; Whatley, 1977; Wooding and Northcote, 1965). In angiosperms the association has been observed in several different organs (including leaves, pollen and secretory tissues), but in almost every case it seems to be limited to a brief period of starch accumulation and loss during early stages of plastid development (Fig. 4).

Pleomorphic Plastids. As proplastids develop into chloroplasts, they regularly go through a phase when they become pleomorphic. This phase can be identified in chloroplasts of species ranging from the alga, *Chara*, to angiosperms and so seems to have been long established. Newcomb (1967) has suggested that the pleomorphic form may represent a "feeding" stage. the semipermeable plastid envelope allows the entry of some selected metabolites and the release of others to the cytoplasm where they may undergo further processing; the pleomorphic form conspicuously increases the plastid surface area over which such exchange of metabolites can take place. Also, in many types of pleomorphic plastid, the normal system of invaginations of the inner envelope membrane and its associated vesicles proliferates considerably. This results in a further increase in the potential exchange surface, but in this case, between the plastid stroma and the intra-membrane envelope channel (Whatley and Whatley, 1987). In C₄ plants, the somewhat similar invagination system

called the peripheral reticulum is believed to be important in CO₂ exchange (Gracen et al., 1972). In some secretory cells and in some petals, much of the interior of each plastid is occupied by a complex tubular network (Whatley, 1977, Cheniclet and Carde, 1985; Brett and Sommerard, 1986) which may, in part, be assembled from vesicles derived from the plastid envelope. The precise forms of these tubular networks vary considerably, but in general they resemble the "thylakoid plexes" (assemblages of thylakoid or tubular elements) described by Spurr and Harris (1988). In plastids with such plexes the capacity for storage of metabolites within the tubules, and perhaps also for their transfer to the envelope membrane space, must be considerable.

Pleomorphic plastids with many inner membrane invaginations and vesicles are particularly well-developed in cells engaged in the secretion of an unexpectedly wide range of unrelated substances, from salt, sugars, and digestive enzymes on the one hand to essential oils, lignin, gums, and resins on the other (Whatley and Whatley, 1987). Pleomorphic plastids are found in many different types of cell (Figs. 5, 13, 14, 15, 16 and 17); in transfer cells of some moss sporophytes, in cotyledon bundle sheath cells in *Welwitschia*, in the mucilage-secreting cells of the *Isoetes* Ligule and of root caps, in epidermal cells of petals and in a wide variety of secretory cells (Paolillo, 1962; Fahn, 1979; Whatley, 1983a; Werker and Zamski, 1985; Whatley and Whatley, 1987; Duckett and Renzaglia, 1988). The apparently numerous metabolites of pleomorphic plastids have not, for the most part, been analyzed, nor have their specific roles in secretion been determined.

The only appropriate review of this subject of which I am aware, is a recent one in which plastid structure and volatile compounds were analyzed in specialized secretory structures of 45 species (Cheniclet and Carde, 1985). When the essential oil contained significant amounts of monoterpene hydrocarbons or oxygenated compounds, the plastid stroma was homogeneous and lacked ribosomes and thylakoids. In the absence or near absence of monoterpenes, ribosomes were present in an electron dense stroma which all contained some thylakoids and a well-developed tubular complex. The published micrographs suggest that most of the plastids are pleomorphic. The authors report a quantitative relationship between plastid volume and the percentage of monoterpenes in the oil, but no such relationship for that of the sesquiterpenes, phenylpropanoid or aliphatic compounds. Nevertheless, they suggest that, in some species, the involvement of plastids in sesquiterpene biosynthesis cannot be ruled out. No

comparable information is available about the possible role of plastids in the biosynthesis of other secreted metabolites.

Chloroplasts. In contrast to the non-green plastids, the chloroplasts of land plants appear to have undergone little structural or other modification (Fig. 3). Nevertheless they do show consistent quantitative variations from one type of cell to another, in their size and numbers, in the extent of their thylakoid systems and in the number and size of their starch grains. Although all chloroplasts follow the same basic pathway during their development, those in each cell file in a root or a leaf, say, will show characteristic quantitative differences. In leaves, for example, spongy mesophyll cells tend to have larger plastids with bigger franal stacks and much more starch than palisade cells and the plastids in the epidermal layers tend to be fewer and smaller and to have a less extensive thylakoid system.

The only major modification in chloroplast structure is in the dimorphic plastids in some C_4 plants, in which the bundle sheath chloroplasts accumulate large amounts of starch and thylakoid system is reorganized (Kirt and Tilney-Bassett, 1978). As indicated above, the development of a peripheral reticulum has been equated with an increase in capacity for CO_2 exchange. The well-known C_4 syndrome appears to be restricted to angiosperms where it has been found in some 500 species from phylogenetically diverse angiosperm families (Downton, 1975). CAM plants (Crassulacean Acid Metabolism) resemble C_3 plants in that they, too, store CO_2 temporarily as organic acid; in both types of plant, modification in the mode of photosynthesis is thought to be an adaptation to an environment where water is a limiting factor. However chloroplasts of CAM plants show no consistent structural modification. Most CAM plants are angiosperms, but this form of metabolism has also been found in *Isoetes* and *Welwitschia* (summarized in Whatley, 1983b).

Responses to Light. Light of different wavelengths is required not only for chlorophyll synthesis, chloroplast development and photosynthesis, but also for many other plant responses. The wavelengths of light received by a particular cell are obviously influenced by both the quantity and the wavelengths of the light absorbed by overlying tissues. The evolution of new pigments, synthesized partly or entirely by the plastids, has accompanied the evolution of new responses to light controlled both directly, by the plants themselves and indirectly, by way of animal vision. It has been suggested that the evolution of

glycoflavones was important initially in providing a screen against ultra-violet light. In ferns have the specific growth responses to blue light developed in response to the evolution of their flavonoids? True anthocyanins are common in angiosperms but elsewhere have been found only in the receptacles of *Podocarpus* and the cones of some other conifers (Swain, 1975). Do anthocyanins in pine cones attract insects as they do in angiosperms?

The prevailing pigmentation of different cell layers within an organ at, say, different seasons can have important developmental or ecological consequences. In citrus the developing seeds are deeply embedded in the fruits, but it is not unusual for the young cotyledons to turn green. In lemons, a large proportion of the light received at the surface is absorbed by the carotenoids and flavonoids of the peel and internal tissues. The wavelengths of light which are not absorbed by the parent fruit, and which in consequence reach the seeds and their embryos, are those very wavelengths which are necessary for the conversion of protochlorophyll to chlorophyll; the quantity of light received is low but nevertheless adequate for the essential photochemical step (Whatley and Price, 1983). In organs where the overlying tissues contain other pigments, different responses will be induced. Of particular ecological importance is the absorption of light by the photosynthetic pigments and its effect on the resulting transmission of the red and the far-red wavelengths to the cells below. Thus, although covered over by many layers of green scales or young leaves, cells in meristems, in dormant buds and seeds, and in pre-flowering shoot apices can all respond to photoperiodic and other phytochrome-based controls (Vince-Prue, 1975).

CONCLUSIONS

From unicellular green algae to angiosperms, photosynthesis has remained the principal function of the majority of plastids, the chloroplasts. Changes in plastid structure and function have almost all been confined to non-green plastids; most have involved modifications of structural components and biosynthetic pathways already present in algal plastids. Though few such changes are found in the algae themselves or even in lower land plants, their introduction may have been linked to the evolution (a) or proplastids in meristems, (b) of multicellular plants requiring conductive tissues and (c) of adaptation to the often dry and unpredictable land environment and to the limitation of predation.

As land plants have evolved, the tendency towards the elimination of comparatively few "waste products" from the non-

specialized cells seems to have become modified. Instead an increasing range of tissue-specific, specialized secretory products are accumulated in localized groups of cells (Fig. 16) as well as in a range of precisely formed cavities and ducts; other such products are ejected from distinctively organized trichomes, glands and other such "new" structures. In lower land plants these cells or structures are uncommon (and the form of their plastids is generally not known); the few recorded examples include mucilage-secreting cells or ducts in some bryophytes and in *Lycopodium*, *Selaginella* and *Isoetes*, hydathodes in *Equisetum*, extrafloral nectaries (some "non-structural") in a few fern genera, salt-, lemon-scented- and flavonoid-secreting-glands in some other ferns and latex-producing cells in the fern, *Regnellidium* (Darwin, 1877, Johnson, 1937; Chapham, Tutin and Warburg, 1962; Smith, Craig and Santarosa, 1971; Horner *et al.* 1975; Bruce, 1976; Fahn 1979; Duckett and Renzaglia, 1988. By contrast, glands and other secretory structures with modified plastids are common in the angiosperms and the evolution of flowers has led to even greater changes in plastid structure. It is particularly unfortunate that almost no information is available about plastid structure in the secretory cells of ferns, as it is there that important clues to the progress of plastid evolution in land plants may well be waiting to be found.

Secretory substances to which plastids are believed to contribute (Kirk and Tilney-Bassett, 1978) are phenolics (lignin, tannin, anthocyanins and flavonoids), isoprenoids (carotenoids, essential oils, terpenoids, resins, lates and the hormones, abscissic acid and giberellin), sugars (especially mucilages and nectar) and lipids (cutin, suberin and waxes). The sugars released as the Stage 2 amyloplasts develop into the Stage 3 pleomorphic plastids may additionally provide an energy source for further synthesis within the endomembrane system. During the evolution of land plants, the major shift in plastid structure and function seems to have been the increased exploitation of the various sub-forms of pleomorphic plastid. These plastids have become characteristic of many different types of non-photosynthetic cell in which they have apparently acquired two important roles; 1) by exploiting existing biochemical pathways, they seem to have extended their biosynthetic capacity and to have become a major source of precursors for an increased range of secondary metabolites and 2) by greatly increasing their surface area and intra-membrane spaces in several different ways, they have increased their potential for the exchange and storage of these metabolites. It therefore seems reasonable to consider pleomorphic plastids not only as major sites for secondary

metabolism, but also as the organellar equivalent of transfer cells (Whatley and Whatley, 1987)

Plastids have many different functions. In addition to their photosynthetic activities they can contribute to metabolites which protect the plant against desiccation and other environmental hazards, and against predators; they contribute to some growth promoting, growth inhibiting, and recognition substances; they provide a range of attractants for insects and other animals which are essential for successful plant reproduction and long-term survival. The pigments to which they contribute modify light in such a way as to allow the operation of photoperiodic and other control mechanisms which permit a form of fine-tuning to the local climatic conditions. The chloroplast, through photosynthesis, has always had a major ecological role as a primary food source. In modern land plants and particularly in angiosperms, many of the varied forms of non-green plastids have also acquired features of significant ecological importance.

SUMMARY

Plastids are believed to have evolved from algae that were once free-living but later were taken up as endosymbionts by unicellular protists. In their new habitat, the photosynthetic symbionts provided their (phagocytic) hosts with new and continuing source of food, but lost many of their other synthetic activities, and much of their control was taken over by the cell nucleus. Once the symbionts had become established and transformed into chloroplasts, there seems to have been a lengthy phase of evolution of green algae and land plants during which there was little change in chloroplast structure and function; the basic cup-shape is retained by many green algae and some lower land plants. However, as multicellular organisms evolved, there appears to have been an increase in the proportion of the plant devoted to a nonphotosynthetic function. The nonphotosynthetic cells virtually all retained their plastids but in a dedifferentiated state. Most subsequent changes in plastid structure and function seem to be associated with these nongreen plastids. Some of them are proplastids in meristems. Others, specializing in the synthesis or accumulation of a range of storage products or secondary metabolites, represent diversions from the basic pathway of chloroplast development; it is these that show the most conspicuous structural modifications. Lower land plants have few such modified plastids; those that do occur may reflect adaptation of the plant to the land environment. Most of the "new" forms of plastid are to be found in angiosperms and many of these seem to

be associated with specialized functions linked to interactions between plants and animals.

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ADAPTIVE SHIFTS TOWARD HUMMINGBIRD POLLINATION

G. Ledyard Stebbins

INTRODUCTION

I take great pleasure in dedicating this contribution to one of my oldest and most revered scientific friends, Herbert G. Baker. For a generation, he has set the example of how to exploit facts extracted directly from nature to illustrate and explain general principles of evolution.

One of the least understood of the major features of evolution is the nature and explanation of events that lead to striking innovations or anagenetic events; those that initiate entirely new directions. One reason for this relative lack of understanding is that such events are rare, and many of them are unique turning points in the three billion year history of organismic evolution. Hard data on these unique events cannot be obtained, since they all occurred in the distant past, and the conditions that brought them about will always remain to a certain extent speculative. An indirect way of approaching the problem is to analyze as carefully as possible intermediate situations; those that are unusual with respect to the origin of the great majority of species belonging to a particular group, but are still common enough, and have occurred recently enough so that the events that brought them about can be reconstructed with reasonable confidence. Particularly favorable are situations involving differently adapted species that are sympatric or nearly so and can be crossed so that segregating progeny from hybrids can be analyzed. When such situations exist, analysis is possible at all levels, from the ecological problem of organism-environment interactions through the problems of conventional cytogenetics, such as the number of gene differences involved, down to the molecular developmental problem of how the genes produce their effects.

Another circumstance favorable for this intermediate, indirect approach would be the presence of several similar examples in the same geographic region. This permits us to test on other examples hypotheses that arise from the first situation to be examined. In no case can we expect to prove conclusively that an hypothesis concerning past events is valid. Nevertheless, the larger is the number of similar but unrelated examples that fit a particular hypothesis, the higher is the probability that this hypothesis is correct.

To botanists living in the western United States, a problem of immediate importance that meets this criteria is the shift in several unrelated genera from adaptation to pollination by Hymenoptera (bees or wasps) or occasionally from Lepidoptera (butterfly) pollination, to pollination by hummingbirds, Trochilidae. This topic has been explored by several botanists. An excellent review by Karen and Verne Grant (1968) is the source of much of the data presented below.

PARALLEL SHIFTS TO HUMMINGBIRD POLLINATION

The Grants list as adapted to hummingbird pollination 129 species belonging to 39 genera and 18 families. Of these species, 21 do not have in California related species that are pollinated by Hymenoptera, so that modern counterparts of the species involved in the adaptive shift are not available, and their evolution must be investigated elsewhere, if at all. This leaves 108 species belonging to 30 genera as favorable material for at least partial analysis. Although in most of these genera only one shift per genus is clearly indicated, the shift probably took place twice in *Delphinium*, *Lilium*, and *Mimulus* (incl. *Diplacus*), and three times in *Penstemon* (incl. *Keckiella*). In the western American flora, therefore, reasonably good evidence exists for 33 separate shifts from hymenopterian or lepidopterian to hummingbird pollination. If other areas, particularly tropical America, were considered, the number would be much larger. For eleven examples: *Aquilegia*, *Delphinium* (2), *Mimulus* (2), *Penstemon* (2), *Gilia splendens*, *Lonicera involucrata* and *Lilium* (2), exchange of genes via hybridization between hummingbird-pollinated species or varieties and those pollinated by insects is clearly possible, and for several other examples it is highly likely. Evolutionists, therefore, have available a rich source of material for exploring this important problem.

THE SYNDROME AND ITS VARIANTS

As a descriptive model of the syndrome, the brilliant analysis by E.O. Guerrant (1982) in Dr. Herbert Baker's laboratory of the red larkspur, *Delphinium nudicaule*, serves ideally. This species grows in moist canyons and stream banks in northern California. It is closely related to a series of partly sympatric species centering about the purple-flowered, bee-pollinated *D. decorum*, between many of which, including *D. nudicaule*, natural hybrids have been recorded. Chromosomally, all are diploid, having the somatic number of $2n = 16$.

The differences between *D. nudicaule* and its relatives are of three kinds, each of which is probably governed by a different

gene system. First, the leaves of *D. nudicaule* are less deeply lobed than are those of its relatives, and are completely glabrous, whereas those of its most nearly sympatric congener, *D. decorum*, are somewhat pubescent. Where these species are sympatric in the northern Coast Ranges, *D. nudicaule* favors moister habitats, grows most actively in the moister weeks of early spring, and blooms earlier than *D. decorum*. Second, the red flower color of *D. nudicaule* contrasts sharply with the dark blue-purple of *D. decorum*. Third, the non-spurred sepals of *D. nudicaule* are smaller than those of its relatives, and are directed forward so that they form a continuation of the nectar bearing floral tube formed by the sepal spur. Guerrant (1982) showed that these sepals grow more slowly compared to the rest of the flower than do those of *D. decorum* and characterizes their growth as neotenic.

Nearly all hummingbird-pollinated species are perennials. A few are shrubby, but the majority are herbaceous. Annuals are exceptional as hummingbird flowers, the only example of the shift from insects to hummingbirds in the western flora being *Gilia splendens*. Hence, in later sections of this review that estimate the probability of an insect-pollinated group giving rise to a hummingbird flower, only perennial species are considered. Hummingbird flowers usually produce large amounts of nectar, in which sucrose predominates, while amino acids, if present, exist in low concentrations (Baker and Baker, 1982). This shift in nectar production is apparently accomplished so easily by genetic change that it is only a minor barrier to the completion of the adaptive shift.

The three major components of the syndrome [shift in vegetative characters that control adaption to habitat, in flower pigmentation, and in floral structure,] have all been involved, via parallel changes, in most of the examples to be considered. This shows that the shift cannot be brought about by one or two mutations, since the genetic basis of these three components is certainly different, as is borne out by the results of hybridization experiments to be reviewed below. In each of them, three different kinds of selection pressures must have been involved: (1) vegetative adaptation to habitats in which hummingbird pollinators are more abundant, at least seasonally, than are large bees or other pollinators to which the ancestors are adapted; (2) a shift in color that makes the flowers more conspicuous and easily approached by hummingbirds than are the ancestors; (3) a floral structure that fits the hummingbird beak better than the insect proboscis.

Nevertheless, some of the examples reviewed by Grant and Grant include only one or two of these three elements. Most often,

the habitat differences does not appear to be pronounced, at least without carefully collected data on the species involved. This is true for *Monardella macrantha* vs. *M. nana*, *Trichostema lanatum* vs. *T. parishii*, *Silene californica* vs. other western American species of the genus, *Astragalus coccineus* vs. other related species, *Fritillaria recurva* vs. *F. lanceolata*, and both *Lilium maritimum* and *L. parvum* vs. their nearest relatives.

Intermediate or less pronounced differences with respect to flower color and structure are less frequent. Color differences in *Castilleja* are usually pronounced, but in at least two examples they are less so. These are the hummingbird flower of *C. Payneas* vs. the insect pollinated *C. pilosa* in northern California, and the hummingbird (probably) *C. coccinea* vs. insect pollinated *C. pallida* and *C. septentrionalis* in northern United States and Canada. In *Lilium parvum*, an entire spectrum of color differences exists from orange-yellow to pinkish red. This may reflect a difference within the species of the most common pollinators, from Lepidoptera to hummingbirds. In their review, the Grants include a photograph of a hummingbird pollinating a normally Lepidopteran flower, *Lilium humboldtii*. With respect to flower structure, there is little if any difference between the corolla of typical *Lonicera involucrata*, that is insect (probably Lepidopteran) pollinated, and its var. *ledebourrii*, that is pollinated by hummingbirds.

Apparently, many flowers are visited by vectors other than the predominant one, but their occasional visits have little or no effect on selection for the adaptive syndromes.

These partial and intermediate situations illustrate two important points. First the shift from insect to hummingbird pollination is not necessarily saltational, although, given strong enough selection pressures, it could be rapid in terms of the geological time scale. Intermediate situations, in which occasional pollination by either of the two vectors is possible, may be a normal transitional stage. Second, the completion of the three parallel trends of selection, giving rise to fully developed differential syndromes, is unlikely to occur in sympatry. The striking differences observed in *Delphinium*, *Aquilegia*, *Mimulus*, *Penstemon* and other genera were probably at least completed, if not initiated, when the hummingbird pollinated species were completely isolated spatially from pollinators carrying pollen from the insect pollinated ancestor.

THE IMPORTANCE OF ECOLOGICAL DIFFERENCES

The Grants have presented a thorough account of the geographical distribution of breeding hummingbirds and of

hummingbird-pollinated flowers. The diversity of habitats is great, but some habitats in the western USA are devoid of both breeding hummingbirds and of hummingbird flowers. These are principally seashore, marsh and aquatic habitats, sagebrush and savanna. Areas that hummingbirds visit but in which they do not breed are lacking or poor in hummingbird flowers. This suggests that hummingbirds have been able to favor the occurrence and frequency of those plant species that bear flowers which they visit, but only if the selective pressure that they exert is continuous, being associated with breeding and raising young. Moreover, the examples that they present show that species which probably evolved adaptations to hummingbird pollination a long time ago, such as *Fouqueria splendens* (ocotillo) and various species of paintbrush (*Castilleja*), are less strictly restricted to breeding sites than are species which are closely related to insect-pollinated relatives. This topic is developed further in a later section.

These ecological relationships show first that natural selection for hummingbird pollination involves adaptation to hummingbird habitats, and increases the probability that, as already stated, hummingbird flowers evolved in geographic and ecological isolation from insect pollinated ancestors.

THE SIGNIFICANCE OF FLOWER COLOR DIFFERENCES

Red coloration of flowers and nearby reproductive organs is characteristic of hummingbird pollinated flowers occurring in western North America. Exceptions occur in other parts of the range of Trochilidae.

The extent of red coloration varies greatly from one species to another. The majority of genera are those having either only the corolla or both corolla and calyx pigmented. In some, (*Monardella*, *Salvia*, *Ribes*, *Epilobium* sect. *Zauschneria*) the pigment extends to the floral bracts or calyx tube. In two species the reddish or pinkish color is more restricted. In *Lonicera involucrata* var. *ledebourii* it is confined to the floral bracts and in *Trichostema lanatum* it occurs only on the hairs that densely cover the inflorescence. The genus *Castilleja* is remarkably variable in this respect. In the majority of its species, red color exists on the tips of floral bracts, calyx lobes, and the margins of the corolla. In some, however, (*C. hololeuca*), the corolla lacks red color, in others, (*C. Lemmonii*, *C. Culbertsonii*), the floral parts are pinkish or purplish rather than scarlet. In still others (*C. Payneae*, *C. pilosa*, *C. nana*) both the extent and hue of color vary among floral organs and parts of them, in still other species (*C. longispica*, *C. arachnoidea*, *C. mollis*, *C. plagiotoma*) reddish or

purplish pigmentation is completely lacking from the floral parts. These color deviants include species in which floral structures closely resemble those of the red flowered species plus others that, with respect to both calyx and corolla, approach the insect-pollinated genus *Orthocarpus*, and were included in this genus by some botanists. The possible evolutionary implications of this situation are discussed below.

The significance of red coloration in bird-pollinated species has been the subject of much controversy, well reviewed by the Grants. Several experiments have shown that the somewhat naive and anthropomorphic hypothesis, that hummingbirds "prefer" red flowers is incorrect. Like many other animals, these birds establish associations between visual cues and sources of food. The Grants, in my opinion correctly, assume that such associations are most easily made if all flowers, regardless of taxonomic affinity, that have structures and nectar supplies suitable to hummingbirds are similarly colored. But why red, rather than yellow, white, pink, or any other color? In this connection, one must note the fact that red is the predominant color not only in New World species pollinated by hummingbirds, but also in Old World plant species visited by birds having very different affinities (Meeuse 1961). Three reasons can be deduced. First, red or scarlet, though found in insect-pollinated genera, such as *Papaver* and *Lychnis*, is uncommon among them. Hence the association of red with structural suitability is a distinctive one for birds and avoids the confusion that would exist if a more common color were associated. Wright (1979), reviewing many experiments on visual sensitivity in the pigeon, says that it resembles humans in the long wave length end of the spectrum. To birds, therefore, red is a conspicuous color as it is among humans. Once the association, red with a source of food, was firmly established, the bird could detect this source more easily from a distance. I and many other naturalists have been amazed by the speed with which a hummingbird will "dive-bomb" a flower of *Castilleja* from ten or scores of meters away. Finally, many birds, including hummingbirds, possess red signals in their plumage that either enable males to advertise and defend their territory more easily, or females to locate more easily a potential mate. Once a bird is conditioned to associated red with various adaptively advantageous behaviors, the association between red and a source of food could, presumably, be more easily made.

CHEMICAL BASIS OF FLOWER COLOR DIFFERENCES

The chemical basis of flower pigments is somewhat complex, and has been extensively reviewed (Harborne, 1964, Goodwin,

1976). The principal compounds involved in those species that have given rise to hummingbird pollination are carotenoids and anthocyanins. Carotenoids are usually found in the chromoplasts embedded in the cytoplasm of epidermal cells or hairs of the corolla, while anthocyanins are dissolved in the vacuoles. The carotenoids are responsible for yellow colors, while the three principal anthocyanidins, delphinidin, cyanidin and pelargonidin, are responsible for colors ranging from blue to purple, lavender, pink and red plus various intermediate shades. Pelargonidin has been identified as the basis of red color in *Mimulus* (Pollock et al., 1967) as well as *Cantua*, *Collomia* and *Ipomopsis* in the Polemoniaceae and *Penstemon* in the Scrophulariaceae (Harborne and Smith 1978). Delphinidin and cyanidin occur also in these families and many more; they are among the most ubiquitous phenolic compounds in angiosperms. Hence, although precise identifications of these compounds have not been made in species believed to be related to ancestors of hummingbird flowers, the fact that all of these postulated ancestral groups have flower colors normally produced by delphinidin or cyanidin suggests that one or both of these compounds were present and a prerequisite for the evolution of hummingbird flower colors. The three compounds have very similar formulae, the ring structure characteristic of flavone (Harborne, 1964, p. 89) unmethylated, and with hydroxyl (OH) groups at various positions. The three differ in that delphinidin is the most hydroxylated (OH at 6 different positions), cyanidin next (5 hydroxyl groups) and pelargonidin the least (4 OH groups). This difference is of evolutionary importance, since genetical experiments on several genera have shown that alleles coding for the more hydroxylated anthocyanidins are dominant over those that code for less hydroxylated compounds (Alston, 1964). The above statement explains only part of a complex genetic picture, that is discussed more fully in a later section.

THE NATURE AND SIGNIFICANCE OF STRUCTURAL DIFFERENCES

With respect to the structure of the flowers and inflorescence, hummingbird-pollinated flowers have been said to possess four different modifications: (1) calyx and/or corolla forming a tube that is long enough to accommodate the bird's beak, so that when probing for nectar at the bottom of the tube, the bird brushes its head or the base of its beak against the stamens and stigma of the flower; (2) a thickening of tissues in those parts of the flower most likely to be damaged by the bird's beak; (3) a plentiful supply of nectar, of which the nutritive components

include a low content of amino acids (Baker and Baker, 1982), and (4) flowers solitary or at least separated from each other in the inflorescence, and pendant, the latter position being in agreement with the hovering position of the bird in seeking nectar.

The third prerequisite is far from precise. In fact, the array of inflorescence types represented in the list of hummingbird flowers presented by the Grants includes almost every type except the flat topped umbel as found in the Apiaceae. The largest of genera have either simple or compound racemes. Solitary or nearly solitary flowers occur in *Aquilegia* and *Ribes speciosum*; a cymose inflorescence in *Brodiaea* (*Dichelostemma*) *ida-maia*, corymbs in *Silene*, dense spikes in *Pedicularis densiflora* and *Castilleja* spp., and a capitate inflorescence in *Monardella macrantha*. Apparently, if other factors favor the shift, no type of inflorescence is a serious barrier to the evolution of hummingbird pollination.

The evolution of a suitable floral tube is, however, much more of a problem. None of the relatives or presumed ancestors of hummingbird flowers have flat or bowl shaped flowers. Small, bell-shaped flowers are also lacking among them, as are corollas having very narrow throats, such as those of many Boraginaceae. Among tubular flowers there appears to be a lower limit to tube length, below which modification to a size large enough for the beak is highly improbable. The floral shape that is most easily modified into a suitable tube via gene-controlled repatterning of growth is either broadly tubular or narrowly bell- or vase-shaped. Such flowers are usually pollinated by medium-sized or large bees or wasps, and require the pollinator to enter the flowers when seeking nectar, rather than merely inserting its proboscis.

The conversion to the hummingbird syndrome usually involves lengthening the tube, and shortening the lobes at its apex, particularly those that in the ancestral flower may have served as a landing platform for the insect. Ancestrally bilabiate corollas are sometimes modified by turning back the lobes (Hiesey, Nobs and Björkman, 1971).

The above description applies to the majority of hummingbird flowers, in which the ancestral flowers have united petals and the calyx is not involved in the formation of the tube. The situation in flowers having separate petals or (in Liliales, tepals) is quite different. In them, either the calyx, or a combination of calyx-corolla (tepals) is involved. In *Silene*, *Astragalus* and *Epilobium-Zauschneria* the sepals are united into a tubular calyx in the ancestral flower. In them, the tube is formed by elongation of the calyx tube, but the petals remain, evolve

scarlet color, and serve as attractants. In *Delphinium* and *Aquilegia*, the chief source of the tube is a spur-bearing petal or sepal. In *Fritillaria recurva* and *Lilium* the tepals are relatively narrow and directed forward, so that they form a broad pseudo-tube, and in both ancestral and hummingbird species the stamens and stigmas protrude far enough from the nectar source so that they brush against bird's head. In the last two genera, the modifications for hummingbird pollination are minimal. They are also distinctive in that the ancestral flowers are pollinated not by Hymenoptera but by large butterflies.

The examples in the last paragraph illustrate an important point. Flowers that become adapted to hummingbird pollinator do not converge toward a similar construction. Given a large enough difference in the structure of the ancestral flower, natural selection for adaptation to hummingbird pollination, occurring simultaneously in different unrelated lines, may cause these lines to diverge rather than converge with respect to structural organization.

TAXONOMIC AND GEOGRAPHIC DISTRIBUTION OF SEPARATE HUMMINGBIRD FLOWERS

Although the number of species in the Western American flora that are regularly cross pollinated by insects has not been precisely estimated, it is surely well over 1000, and may approach 2000 species. The 33 separate conversions to hummingbird pollination, therefore, have arisen from not more than 2 to 3 percent of the species that make up the flora. To what extent has this conversion depended upon the chance presence of hummingbirds, and to what extent have been involved either gene-controlled characteristics of the ancestral populations, or certain attributes, many of which probably also have a genetic basis, that have adapted them to habitats in which hummingbirds are relatively numerous compared to other pollinators? The factors that could have caused deviations from chance are here reviewed.

An important point brought out by the Grants is the scarcity of hummingbird-pollinated species among native annuals. They list only one annual, *Gilia splendens*. They attribute the scarcity to the fact that hummingbirds are attracted to flowers that produce an abundant supply of nectar over a long period of time, while annuals usually bloom only for short periods, since they must ripen their seeds before the summer drought. Another drawback to the annual habit is that it is often associated with self-pollination, and self-pollinated flowers tend to be small and produce little nectar.