1979

The Aloineae: A Biosystematic Survey

Herbert Parkes Riley

Shyamal K. Majumdar

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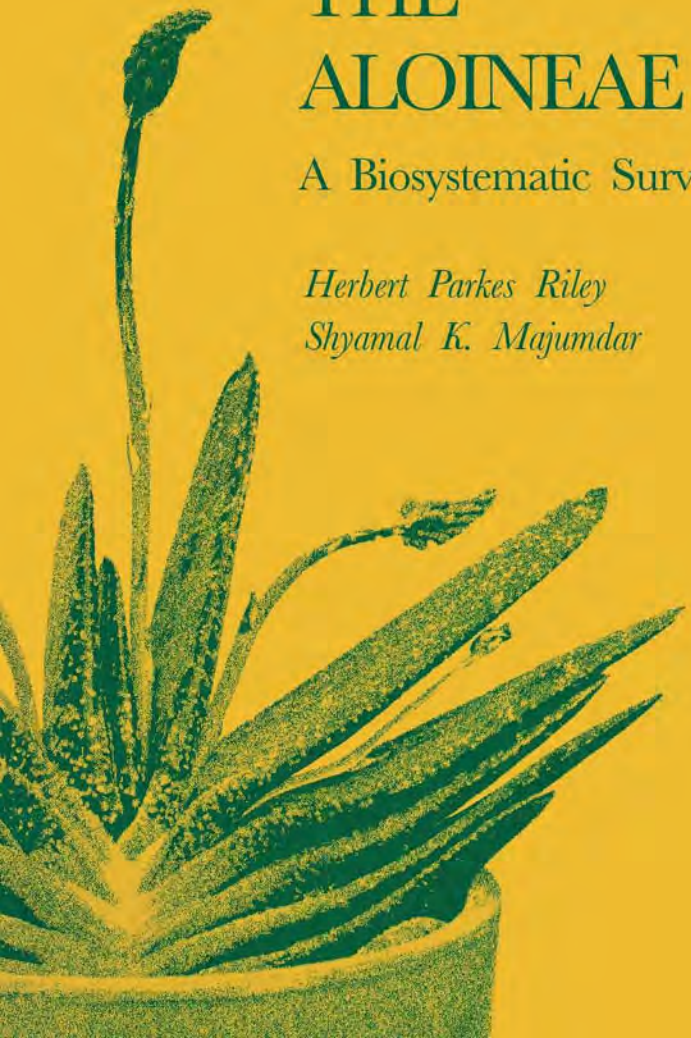

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THE UNIVERSITY PRESS OF KENTUCKY

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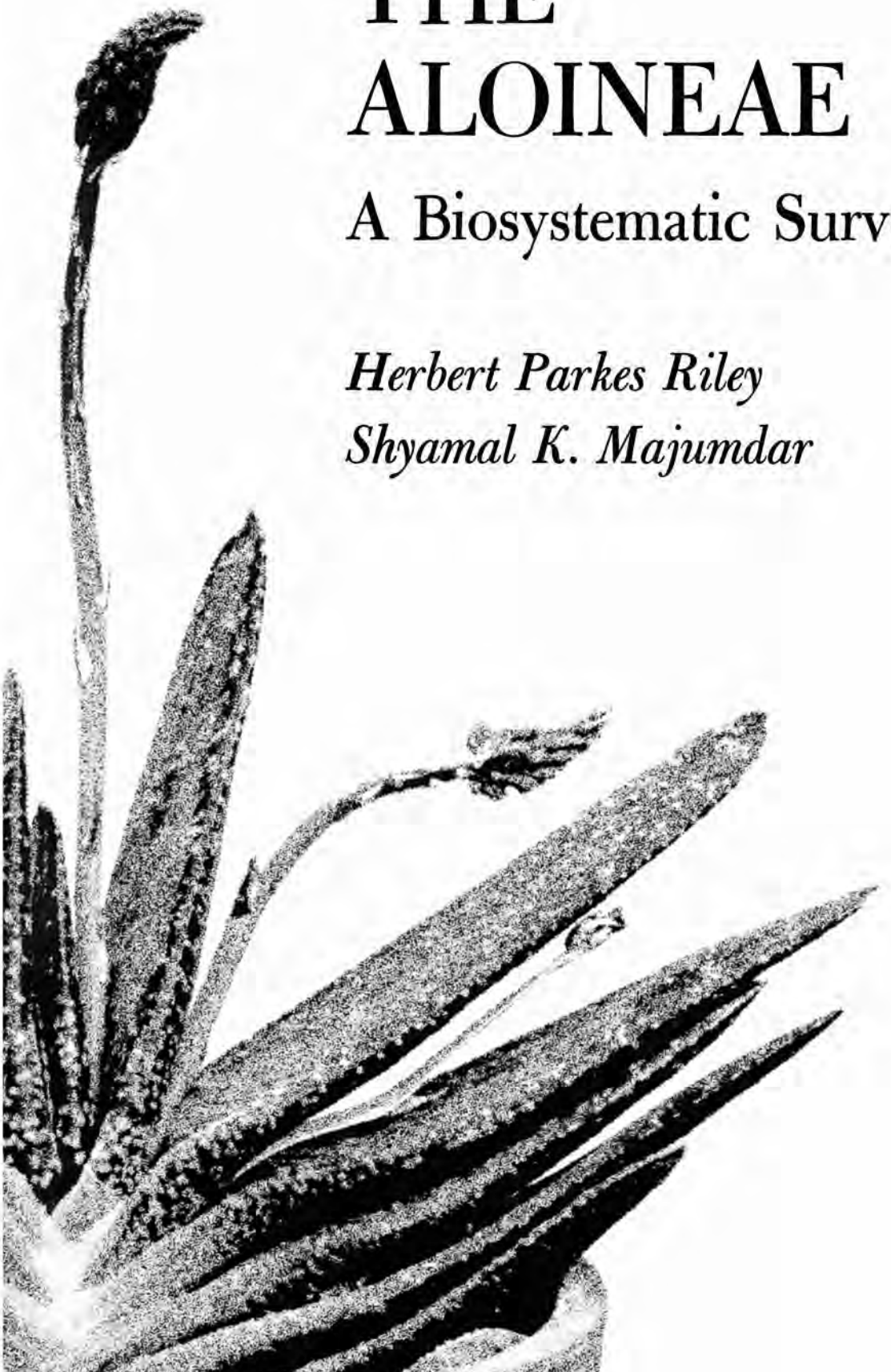

Library of Congress Cataloging in Publication Data

Riley, Herbert Parkes, 1904-
The Aloineae, a biosystematic survey.

Bibliography: p.
Includes indexes.
1. Liliaceae. 2. Plant cytotaxonomy. I. Majumdar, Shyamal K., 1938- joint author. II. Title.
QK495.L72R53 584'.324 77-92927
11.laceae.
1. 1938-

ISBN: 978-0-8131-5592-0

Scholarly publisher for the Commonwealth
serving Berea College, Centre College of Kentucky,
Eastern Kentucky University, The Filson Club,
Georgetown College, Kentucky Historical Society,
Kentucky State University, Morehead State University,
Murray State University, Northern Kentucky University,
Transylvania University, University of Kentucky,
University of Louisville, and Western Kentucky University.

Editorial and Sales Offices: Lexington, Kentucky 40506

Contents

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Preface

During the last fifty years the chromosomes of the Aloineae have been the basis of a number of studies by cytologists and cytogeneticists in many parts of the world. The early studies were concerned with learning about the chromosomes themselves and their structure and behavior during mitosis and meiosis, two processes that were then just beginning to be understood. Later studies were concerned chiefly with the evolution of the Aloineae, especially with chromosome factors involved in their evolution. Much more recently factors other than chromosomal that might have an influence on the evolution or biosystematics of a group of plants have been investigated and are being appreciated; even thirty or forty years ago they were occasionally considered, although not so extensively.

The studies on the Aloineae have been written in English, French, German, Italian, Japanese, Portuguese, and Afrikaans, and have been published in many journals (some rather obscure) in Europe, Africa, Asia, and North and South America. Some of the publications were by us and our associates. The purpose of this book is to assemble in one place all this published material and to try to interpret from the works of the various authors some evolutionary trends and the possible taxonomic significance, if any, of the chromosomal and other factors operating in the development of the group.

The extensive literature on Aloineae has been covered in this book as completely as possible. Only an occasional rare foreign publication has been unavailable; one, first published in Portuguese, was of little concern since the original author later republished it in English in a better-known journal. Nearly all Japanese articles include English or German summaries; the only one that did not was primarily a list of chromosome numbers of various species of plants and therefore largely useful, even though neither of us is competent in even elementary Japanese. The most important paper on the chromosomes of *Aloe* is the Ph.D. dissertation of F. S. Müller, which was published in Afrikaans. This language is little known in the United States but is easy to read; it is basically a simplified form of Dutch, with practically all the declensions and conjugations eliminated, and has many words that are cognate forms of English or German words and therefore easy to assimilate.

The senior author wishes to express his gratitude to many people and several organizations in South Africa and

the United States who have been helpful in the conduct of his research in the Aloineae and/or in the preparation of this summary. The late M. R. Levyns, formerly a senior lecturer in the Department of Botany in the University of Cape Town, and her husband, J. E. P. Levyns, provided inspiration and valuable suggestions and spent much time showing him botanical features of the landscape of the western Cape Province. Professor J. D. J. Hofmeyr, now retired from the Department of Genetics of the University of Pretoria, provided him space and research facilities during his trip to South Africa over twenty years ago. Other South Africans who were helpful primarily in supplying seeds or live plants of the Aloineae were A. Berg of the University of Pretoria, Harry Hall of Kirstenbosch, and Hans Herre of the University of Stellenbosch.

Several organizations were particularly important in furthering this study. First and foremost was the Fulbright committee that provided the senior author the opportunity to visit South Africa in 1955-56 and to collect material, observe the plants in their natural habitats, and make contacts that enabled him to continue the work later. More recently, in 1963, an invitation to join in the celebration of the Golden Jubilee of the National Botanic Garden of South Africa and to spend a month on a conducted tour of the whole country enabled him to continue the observations he had made previously and to spend considerable time studying the collection in that fabulously beautiful garden. To Professor H. Brian Rycroft, the director, he is very grateful. Indirectly the governments of the Union of South Africa in 1955 and its successor, the Republic of South Africa, in 1963 helped by providing him the necessary visas and permits to enter and study in their fascinating country.

Grants from several organizations provided funds that really made this study possible. They include the Haggin Scholarship of the University of Kentucky and a National Science Foundation summer fellowship to the junior author. Grants from the Program of Genetic Biology and the Program of Systematic Biology of the National Science Foundation, from the Joseph Henry Fund of the National Academy of Sciences, and from the University of Kentucky Research Foundation were given to the senior author.

Leaves of absence to visit and study in other countries are rarely granted as a matter of right but usually as a privilege. Therefore, the senior author is especially appreciative of three men who were responsible for his two leaves of absence to go to South Africa: Herman L. Donovan and Frank G. Dickey, both former presidents of the University of Kentucky, and M. M. White, dean emeritus of the College of Arts and Sciences.

For many years the authors and their co-workers have been studying various aspects of this group of plants. We especially mention Debdas Mukerjee, who contributed much to the study of the Aloineae.

Several authors, scientific journals, and publishers have given us permission to reproduce or redraw various illustrations from their published works. Specifically we would like to mention Peter E. Brandham of the Jodrell Laboratory of the Royal Botanic Gardens at Kew, Gordon D. Rowley of the Agricultural Botany Department of the Uni-

versity of Reading, the *Journal of South African Botany,* the *Canadian Journal of Genetics and Cytology,* the *Journal of Heredity, Chromosoma, The Nucleus* (India), *Advancing Frontiers of Plant Science* (India), *Grana, 30th Annual Proceedings of the Electron Microscopy Society of America, Pyton, The American Biology Teacher, Taxon, Plant Systematics and Evolution,* the Ronald Press Company, and John Wiley and Sons, Inc. These permissions are gratefully acknowledged.

Herbert Parkes Riley
Shyamal K. Majumdar

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Chapter One

INTRODUCTION

The tribe Aloineae of the family Liliaceae is fundamentally a South African group, but some of the genera included in it are also found elsewhere. It is rather small and is variable, since not all authors include the same genera within it. As treated here the tribe will include *Aloe, Gasteria, Haworthia, Astroloba (Apicra), Poellnitzia, Chamaealoe, Chortolirion, Lomatophyllum, Leptaloe,* and *Guillauminia.*

The Aloineae comprise a group of generally succulent plants characterized by having a loculicidal capsule and anthers with introrse dehiscence. The leaves are generally thick or fleshy and often toothed on the margins; they often have a bitter sap that sometimes is used medicinally; and they contain a soft, green, water-storing tissue. The rootstock is a rhizome and is not bulbous, as it is in most of the Liliaceae. The flowers are generally in a raceme or panicle, and the segments of the perianth are connivent or united into a tube at the base.

Aloe Linn. (Sp. Pl., 1753, p. 319) is a large genus, is the most widely distributed geographically, and is probably the best known. The plants may be herbaceous and about 23 cm tall (as in *Aloe myriacantha*) or arborescent and about 15 m tall (as in *A. eminens*) and may be stemless or caulescent; if stems are present they are simple or branched. The leaves are fleshy and succulent and many-ranked. They may be toothed or prickly on the margins. They are usually crowded in a dense rosette, which is acaulescent and on the ground or may occur at the end of a short or long stem (Figs. 1.1-1.5). The flowers are in a raceme or an unbellate or subcorymbose panicle and occur at the end of a peduncle, which may be of considerable length. The perianth is subcylindrical and straight or slightly recurved, in a campanulate or cylindrical tube; the segments are elongated, much imbricated, and spread at the tip if at all (Fig. 1.6). There are six hypogynous stamens, equaling or exceeding the perianth, with subulate filaments; the anthers are small, oblong, and dorsifixed, and dehisce introrsely. The oblong ovary is sessile and somewhat trigonous, with many ovules. The style is filiform and the stigma is small and capitate. The fruit is a coriaceous trigonous capsule, loculicidally three-valved. Seeds are flattened or three-angled, often winged; the embryo is straight and the endosperm fleshy. The flowers are generally numerous, usually bright red or yellow, and usually beautiful. The genus occurs in southern and trop-

Opposite page: 1.1 *Aloe dichotoma;* 1.2 *Aloe speciosa;* 1.3 *Aloe davyana;* 1.4 *Aloe marlothii.*

Above: 1.5 *Aloe ramosissima.*

Below: 1.6 Typical *Aloe* flowers.

1.7 *Gasteria fasciata* (left) and *Gasteria conspurcata*.

1.8 Gasteria species.

1.9 Typical *Gasteria* flowers.

ical Africa, Madagascar (Malagasy), the Mediterranean region, and much of Asia Minor, and has been reported from India.

Gasteria Duval (Pl. succul. in Horto Alençonio, 1809, ex Haw., Syn. Pl. succul., 1812, p. 85) is a large genus, although fewer species have been reported for it than for *Aloe*. The stem is usually absent; if present, it is short. The plant is never arborescent as are some species of *Aloe;* therefore, the plants are small or medium-sized. The leaves are thick and fleshy and are distichous or multifarious, although the latter condition may vary with age; some plants that are distichous when young become multifarious and form rosettes as they get older. The leaves are usually spotted with white, although in some species the spots are not distinct. The leaves are never toothed and are often flecked with warts either on the margins or on the surface (Figs. 1.7, 1.8). The inflorescence is a lax raceme. The perianth is tubular —

ventricose at the lower part, cylindrical above, and narrowed at the base (Fig. 1.9). There are six hypogynous, slightly declinate stamens with filiform filaments and dorsifixed anthers dehiscing down the face. The sessile, oblong, three-angled ovary contains numerous ovules; the style is filiform with a small capitate stigma. The fruit is a three-valved capsule with many compressed winged seeds. The flowers are red, often with green tips, or may be dull pink. The chief diagnostic character is the swollen ventricose lower part of the perianth, which is responsible for the generic name. The genus is endemic in South Africa.

Haworthia Duval (Pl. succul. in Horto Alençonio, 1809, ex Haw., Syn. Pl. succul., 1812, p. 90) is also a large genus. It consists only of small succulent plants which usually reproduce freely from suckers, forming small clumps. The stems are short or elongated and leafy. The leaves are short, thick, fleshy, and often tubercled; sometimes they have small teeth or less frequently cilia on the margins and keel (Figs. 1.11-1.17). The inflorescence is a simple or panicled raceme with short ascending pedicels. The flowers are small (generally 2 cm or less) and whitish with green or reddish brown ribs. The perianth is apparently a straight or oblong-cylindrical tube; the limb is bilabiate with three subequal segments pointing upward and three pointing downward; the latter are more reflexed than the others. Six hypogynous stamens are present and are shorter than the perianth; the filaments are filiform and the anthers are versatile, dehiscing introrsely. The ovary is sessile and oblong-trigonous with numerous ovules. The style is subulate and the stigma capitate. The capsule is oblong-trigonous, coriaceous, and loculicidally three-valved (Fig. 1.18). The genus is endemic in southern Africa.

Astroloba (*Apicra*) Uitew. (Succulenta, 1947, p. 53) is a small genus of small succulent plants that grow in clumps, much like those of *Haworthia*. The stem is always leafy and elongated; the leaves are short, thick, and fleshy and are multifarious or quinquefarious (Figs. 1.19-1.21). The inflorescence is a simple or compound lax, suspicate raceme with small whitish flowers. The perianth is a straight tube of six short oblong segments and has three green stripes down the keel. The perianth limb has six segments regularly arranged in the form of a star — a characteristic that separates *Astroloba* from *Haworthia*, which has a two-lipped instead of a regular corolla. The six hypogynous stamens have filiform filaments with small versatile anthers. The ovary is sessile and oblong-trigonous with numerous ovules; the style and stigma are as in *Haworthia*. The coriaceous capsule is oblong-trigonous and loculicidally three-valved. The genus is endemic in South Africa.

Poellnitzia Uitew. (Succulenta, 1940, vol. 22, p. 61) is a monotypic genus that closely resembles *Astroloba* (*Apicra*); it has larger flowers that are orange-red and yellow, with a limb formed with six segments joined at one point. The flowers do not open and this character separates the two genera. *P. rubiflora* (= *Apicra rubriflora* L. Bol.) is found in South Africa.

Chamaealoe A. Berger (Engl. Jahrb. 36, 1905, p. 43)

1.10 An intergeneric hybrid, *Haworthia retusa* X *Gasteria obtusifolia*.

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Several Haworthia species:

1.11 *H. reinwardtii* var. *reinwardtii*
1.12 *H. fulva*
1.13 *H. glauca*
1.14 *H. truncata*
1.15 *H. sampaiana* (a clonotype)
1.16 *H. emelyae*
1.17 flowers and inflorescence of *H. browniana*
1.18 *H. setata* var. *gigas*

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is another monotypic genus of herbs with numerous thick leaves that are aggregated into a rosette, ovate at the base and linear above, and toothed on the margins. The small flowers are arranged in simple lax racemes. The perianth is tubular and the segments are free from the base. The inner ones are recurved. The six stamens have filiform filaments and oblong anthers. The ovary is oblong to globular and the style is straight. The only species, *C. africana* (Haw.) Berger (= *Bowiea africana* Haw.), is South African.

Chortolirion A. Berger (Pflanzenreich, 1908, vol. 33, p. 72) is a small genus of four species. The plants are small, perennial, and scarcely succulent, with spirally arranged leaves and globular or oblong bulbs. The leaves are grasslike and narrowly linear, toothed on the margins. The small red or white short-pedicelled flowers are arranged in a lax raceme. The perianth is subbilabiate; the segments are united at the base and form an apparent obclavate-cylindric tube. The three lower segments of the perianth are usually slightly recurved. The six stamens have slightly unequal filaments and oblong anthers that dehisce introrsely. The style is straight and the stigma capitate. Three of the species are in the Transvaal and Botswana and one is in Angola.

Lomatophyllum Willd. (Ges. naturfor. Fr. Berlin. Magaz., 1811, vol. 5, p. 166) is a small Madagascan genus of shrubby or arborescent succulents. The stems are simple, and the fleshy lanceolate leaves grow on the ends of branches and look like *Aloe* leaves. The inflorescence is simple or branched, and the many flowers are arranged in a raceme. The flowers are reddish yellow or orange. The perianth is tubular and straight, ovate around the ovary, then somewhat contracted. Six stamens with linear-oblong anthers are inserted at the base of the perianth. The ovary is globular, the style filiform and long. The capsule is bacciform. The genus is found in Madagascar and Mauritius.

Leptaloe Stapf (Bot. Mag., 1933, t. 9300) is a small genus with six species. The stem is absent; the leaves are rather fleshy, narrowly linear, and spirally arranged, with very small spinelike teeth on the margins. The inflorescence is a contracted raceme. The curved tubular perianth is bilabiate, comprised of segments tightly connivent except at the tips. There are six hypogynous stamens, shorter than the perianth segments but exserted from the throat; the anthers are dorsifixed and the dehiscence is introrse. The sessile ovary is six-grooved and three-chambered; the style is larger than the stamens and bears a minute stigma. The fruit is crustaceous or subcoriaceous, and the dehiscence is loculicidal. The genus is endemic in South Africa.

Guillauminia Bertrand (Cactus Français, 1956, vol. 49, p. 41) is quite distinctive. The plants are small and the leaves are narrow and muricate. The plants form small groups of compact rosettes and have roots that are cylindric rather than fusiform. It is the flowers, however, that are distinctive. They are only about 10 mm long, widely campanulate, about 14 mm across the mouth, and white; the three inner filaments grow much more rapidly than the three outer ones, and as a result the anthers

1.19 *Astroloba dodsoniana*.

1.20 *Astroloba spiralis.*

1.21 *Astroloba congesta.*

are exserted 8 mm. The genus is found only in Madagascar.

One of the distinctive characteristics of the Aloineae is their chromosomes, which are very large and not too numerous. The karyotypes include both long and short chromosomes, and the short ones are much larger than the chromosomes of many other organisms. They are ideal for cytological research and for teaching cytology.

Chromosome studies on plants have been numerous during the last hundred years and especially frequent since about 1926. Before that time the method of studying plant and animal chromosomes was laborious and time consuming, greatly restricting the number of organisms that could be studied. The first great advance in our knowledge of the structure, number, and behavior of chromosomes came about 1926 with the development by John Belling (1926) of the squash or smear technique — a method that was excellent for plants but almost useless for mammals. It was so rapid and excellent that many plants were studied cytologically during the next decade, a large amount of data was assembled pertaining to chromosome structure and behavior, and the new field of cytogenetics was born. In the 1950s Tjio and Levan (1956) developed a variation that could be applied to mammalian chromosomes, and since that time much important work on mammals has been published. From 1926 to 1979 thousands of plant species have been studied, and a good understanding of chromosome evolution has resulted. It has also been found that not all species and not all genera are equally good for cytogenetic studies.

In general the chromosomes of the monocotyledons are large and clear and are brilliantly stained in both root-tip and pollen squashes by the Feulgen technique and with acetocarmine and aceto-orcein. The lily family seems to have exceptionally good chromosomes for study — particularly the tribe Aloineae where, except for polyploidy and chromosome aberrations, the various species have a haploid complement of four long and three short chromosomes. The excellence of the chromosomes for study, the ease of growing the plants, the small size of most of the species, the general availability of the plants, and the ease of preparing the slides have led a number of cytologists to study the various genera and species of this tribe.

Chapter Two

GEOGRAPHICAL AND ECOLOGICAL FEATURES

The Aloineae are a tribe almost exclusively of the southern and eastern hemispheres; only *Aloe* is found outside those areas. A few species get above the equator in the eastern hemisphere, and in the western hemisphere one species has been introduced. The individual species differ considerably in the size of the regions they occupy; a few range rather widely within the confines of those hemispheres whereas many are very constricted and appear to consist of only a few small clumps. The species of this tribe are all succulent plants and therefore inhabit the drier parts of the country.

Aloe L.

The only genus that has an extensive range and the only genus that gets beyond the boundaries of South Africa, its enclaves, and adjacent South-West Africa (Namibia) is *Aloe*. A general view of the area it covers in the eastern hemisphere is shown in Figure 2.1. In southern Africa it is found in all four provinces of South Africa (Fig. 2.2) and in South-West Africa, in Lesotho (formerly Basutoland), Botswana (formerly the Bechuanaland Protectorate), and Swaziland, which are integral parts of South Africa geographically and botanically if not politically.

Aloe grows all over the Cape Province. It is the only genus of the Aloineae that occurs on the Cape Peninsula, where four species have been found. *A. commixta* Bgr. is occasionally found at the tops of the hills near Fish Hoek and Kommetjie growing among bush-covered rocks; it is frequent on hills near Glencairn. Apparently it has never been found elsewhere. (According to Adamson and Salter [1950] it is synonymous with *A. gracilis* Haw. but this statement is undoubtedly an error; according to Reynolds [1950] it is a synonym for *A. gracilis* Bak., non Haw., and this is probably true.) *A. succotrina* Lam. grows above Kirstenbosch and is found occasionally on Constantiaberg and Karbonkelberg and on Table Mountain. It grows in sheltered places on rocks on the mountains and is quite local in area. *A. saponaria* Haw. is found chiefly in the coastal bush between Chapman's Peak and Llandudno, a very small area, with a few plants growing on the hills near

2.1 Southern Africa.

Kommetjie and at Slangkop. *A. arborescens* Mill., according to Reynolds (1950), is the most widely distributed of all the aloes of South Africa. It is not indigenous on the Cape Peninsula but was planted on the Cape Flats north of False Bay as a hedge, from which it has escaped and become established.

Just north of the Cape Peninsula and the adjoining Cape Flats is an interesting area which includes the great wine-growing region of the Cape. It is located primarily between Cape Town and the Hex River Mountains. The region from Cape Town to Stellenbosch, Paarl, Wellington, and Malmesbury to somewhat north of Piketburg is one of fruit and winter crops with cattle and sheep; crops are widely grown on the west side near the Atlantic Ocean and northwards to around Van Rhynsdorp. Some aloes have been found in this area. *A. mitriformis* var. *pachyphylla* grows only near Tulbagh and *A. plicatilis* near Tulbagh, Franschhoek, and Stellenbosch. *A. glauca* var. *muricata* has been found near Piketburg; from Van Rhynsdorp to Clanwilliam is *A. comosa*. *A. krapohliana* is in the Van Rhynsdorp Division and in the Richtersveld and Bushmanland.

North of the Van Rhynsdorp Division the region is drier, with an annual rainfall of 25 cm or less. Most of this region is known as Little Namaqualand; the very northern part, bordering on the Orange River, is called the Richtersveld or, less often, Richterveld. Along the Atlantic Ocean and extending inward 80 to 160 m is a desert area with an annual rainfall of 12.5 cm or less. In Little Namaqualand and from Clanwilliam to the Richtersveld is the famous tree aloe, *A. dichotoma;* it is also

2.2 South Africa and neighboring countries.

found from Pella to Upington and in South-West Africa. It grows up to 8 to 10 m in height, has a trunk about 1 m in diameter at ground level, and branches dichotomously. It has been known since Governor Simon van der Stel's journey to that region in 1685. This tree is called Kokerboom in Afrikaans and was used by the non-European inhabitants of the region to make quivers for their arrows. (Koker is an Afrikaans word for quiver.) Little Namaqualand also includes *A. glauca, A. khamiesensis, A. melanacantha, A. variegata,* and several species that have not been studied by cytologists. *A. karasbergensis, A. pearsonii,* and *A. ramosissima* occur in Little Namaqualand and extend northward to the Orange River, which separates South Africa from South-West Africa at that point. *A. dichotoma* is also found near Clanwilliam, as is *A. framesii; A. variegata* is near Calvinia. *A. distans,* as listed in Reynolds (1950), is found near the western coastline at Saldanha Bay and at nearby Paternoster and St. Helena.

Just east of the Cape Peninsula, from False Bay to Cape Agulhas, is a beautiful coastal region with several small towns. Near there *A. succotrina* grows on the bush-covered hills near Hermanus and on the sea cliffs near Kleinmond; it occurs along the coast to the mouth of the Steenbras River as well as on the Cape Peninsula. (The Cape Peninsula plants have always been considered as being plants of *A. succotrina* but the mainland plants are regarded by Jacobsen and others as *A. purpurascens* Haw.; Reynolds [1950], however, considers that the latter is simply a geographical form of *A. succotrina* and neither a valid species nor a good variety.) In the general area of Caledon are found *A. brevifolia* var. *depressa* and *A. glauca;* a little farther inland *A. arborescens* is found in the Rivier Sonder Einde Mountains and near Genadendal.

East of the Stellenbosch-Tulbagh region is the vast dry area that extends eastward to Grahamstown, Cradock, and Steynsburg and occupies over half of the Cape Province. It includes more than forty of the divisions of the southern and central Cape and has a total area of about 400,000 sq km. This area, the Karroo (or Karoo), covers almost one-third of the area of all South Africa. Four of the southwestern districts of the Orange Free State are sometimes included and the Karroo is sometimes described as extending northward to Bloemfontein; these districts are usually not considered to be part of the Karroo, however.

The word karroo apparently comes from a Hottentot word meaning dry or bare. The actual use of the word is vague, as it sometimes denotes physiographic regions and sometimes vegetative ones. It is a region of low rainfall, bright sunshine, and very dry air. These factors, combined with extremes of heat and cold, are suitable to the xerophytic vegetation that it supports. Both the nature of the Karroo and its boundaries defy precise definition and, as Adamson (1938) pointed out, the term is so general that it cannot be applied strictly to a particular vegetation type or to a definite geographic region.

At the southern end of the continent, along the Indian Ocean, is a narrow coastal belt or coastal plateau that varies in width from 5 to 50 km and averages 200 m in elevation. The Karroo extends inland from this coastal re-

gion and is separated from it by the Langeberg Range and the Outeniqua Mountains. The Karroo is often divided into two or three regions, depending upon the way in which the term is used. At the southern end is the Little Karroo, a rather small region between the Langeberg and Outeniqua ranges to the south and a series of higher mountains running roughly parallel to and north of them. These latter are the Witteberg and Zwartberg ranges. The Little Karroo is about 24 km wide on the average and is about 500 m above sea level. There are a number of small subdivisions of the Little Karroo, such as the Robertson Karroo, but they need not be considered here.

North of the Little Karroo is the Great Karroo. It is generally considered to comprise the plateau found between the Zwartberg and Witteberg ranges in the south and the Roggeveld Mountains, the Komsberg Range, and the Nieuwveld (Nuweveld or Newveld), Sneeuberg, and Stormberg ranges in the north. Beyond these mountains is the Upper Karroo. These mountains are generally higher than the southern mountains, rising to more than 1.8 km above sea level. The average elevation of the Great Karroo is 600-900 m and the region is actually a series of basins formed by the long erosion of rivers flowing at right angles to the Cape Folds. The chief cities of the eastern, central, and western parts of the Great Karroo are, respectively, Cradock, Graaff-Reinet, and Beaufort West. The northern boundary of the Upper Karroo is not clearcut and is often considered to be the Orange River. North of that region is the High Veld. The tableland gradually rises from about 900 m in the Great Karroo to about 1,700 m in the High Veld.

The Little Karroo and the Great Karroo differ considerably in their vegetation. In the former, succulent plants are dominant and shrubs and dwarf trees are numerous. Species of *Mesembryanthemum* and related genera are common. In the Great Karroo, in contrast, succulents and trees are less common; grasses are numerous with respect to species but scarce with respect to the number of individual plants. In many places, especially in the western part of the country, as around Laingsburg, one karroo type merges into another. Many of the karroo plants have a high nutritional value and the region is great sheep country, with many goats also raised there.

Many aloes grow in more than one of these regions of southern South Africa; the boundaries are so indistinct that it is difficult to separate them. In the coastal region near Riversdale and Bredasdorp is *A. brevifolia; A. brevifolia* var. *postgenita* has been collected in the Swellendam-Ashton area. *A. ferox* ranges extensively and occurs in the Riversdale Division as well as in the Karroo and in many other places in South Africa. From Swellendam to Mossel Bay is *A. arborescens*.

A. comptonii, a species named after Professor Compton, who was director of the National Botanic Gardens of South Africa at Kirstenbosch for many years, has been collected from the general area of Uniondale, Willowmore, and Steytlerville westward to Laingsburg and Montagu. Part of this region is east of the Karroo, part is in the Little Karroo, and part is in the Great Karroo. In both the Great Karroo and the Little Karroo is found *A. humilis*

var. *suberecta*, which has been collected at Willowmore and Oudtshoorn. *A. microstigma* occurs in the Great Karroo east of Ceres to Laingsburg, in the Little Karroo from near Worcester and Robertson to Oudtshoorn, and eastward to the Great Fish River Valley, Cradock, and Grahamstown. *A. longistyla* is found from Oudtshoorn and other places in the Little Karroo to Willowmore, Aberdeen, and Graaff-Reinet in the Great Karroo, and on to Cradock and near Grahamstown. Along the Langeberg from the Riversdale Division is found *A. gracilis* var. *decumbens*. A karroo form of *A. glauca* is found just west of Koup in the Laingsburg Division and near there between Matjesfontein and Sutherland. *A. lineata* var. *muiri* occurs between Ladismith and Amalienstein and also in the Riversdale, George, and Mossel Bay divisions; it is also in Seven Weeks Poort and along the north slopes of the Langeberg and the Outeniqua Mountains.

A. mitriformis grows extensively in the mountains of the winter rainfall area of the western Cape Province and extends from the Rivier Sonder Einde Mountains between Swellendam and Bredasdorp north and west to the Bokkeveld Mountains. It has also been found on Bain's Kloof, du Toit's Kloof, near Tulbagh, and around Montagu and Ladismith. The senior author has collected it from the side of a hill about 12 m above the old Franschhoek road. It was growing among the rocks and had a mass of red flowers; plants growing in the sun had redder leaves than those in the shade.

In the Great Karroo aloes are scarce. Of those studied cytologically, *A. aristata* has been found in the Stormberg and Sneeuberg mountains and in the Graaff-Reinet Division; *A. framesii* and *A. variegata* have also been collected near Graaff-Reinet. Aloes are scarce in the Upper Karroo although some are found there.

A. saponaria and *A. leptophylla*, which is considered by Reynolds (1950) to be a synonym of *A. saponaria* although a good species by Jacobsen (1954), have been found at Cathcart, Middleton, and Komga in the area north of Grahamstown, as well as on the Cape Peninsula. *A. microcantha* is found from just east of Grahamstown west to Uniondale. Many of the plants of this species are in the constant rainfall region, which has an annual rainfall of 45-60 cm, and which lies between the winter and summer rainfall areas. *A. grandidentata* ranges extensively from Bethulie in the Orange Free State to Colesburg, Philipstown, and Strydenburg and northwestward to Griquatown and Kuruman; it has also been reported from Hopetown, Kimberley, Barkly West, and Mafeking, all in the northern Cape Province, and in Botswana and the Transvaal.

East of the Little Karroo is a large region with numerous succulent plants, including many members of the Aloineae. This area includes Port Elizabeth, Grahamstown, East London, and the land northward to Queenstown and the Stormberg Range. In the general region around Port Elizabeth have been found *A. africana, A. ciliaris, A. gracilis, A. humilis* and vars. *echinata* and *incurva, A. lineata, A. microcantha, A. myriacantha, A. pluridens, A. speciosa, A. striatula* and var. *caesia* f. *conimbricensis, A. tenuior* vars. *decidua* and *densiflora, A. tidmarshii* (= *A. ciliaris* f. mut. *tidmarshii*), and *A. variegata*.

East of Port Elizabeth and along the Bushmans River in the Alexandria Division is some beautiful and very wild, desolate country with many aloes growing on the hillsides. There the senior author collected a large number of plants, including fifty of a wild population of *Gasteria zeyheri*. Across the road on a rocky hillside with a kranz at the top were hundreds of aloes. *A. ferox* was growing abundantly near the bottom of the hill and *A. speciosa* was near the top and at the top of the kranz. *A. ciliaris* was also in the general area. A few kilometers north of the Bushmans River Poort is the cathedral city of Grahamstown. Near the city have been recorded *A. africana, A. ciliaris, A. ferox, A. gracilis, A. humilis* and vars. *echinata* and *incurva, A. lineata, A. longistyla, A. microcantha, A. microstigma, A. myriacantha, A. pluridens, A. pratensis, A. saponaria, A. speciosa, A. striatula* and var. *caesia* f. *conimbricensis, A. tenuior* vars. *decidua* and *densiflora, A. tidmarshii,* and *A. variegata*.

Still farther east is the Great Fish River, where there are thousands of succulent plants, including many of the genus *Haworthia*. Here is *A. ferox,* a tall, treelike species, usually 2-3 m high but sometimes up to 4-5 m and densely covered with the remains of old, dried leaves. Each plant bears one panicle, which branches and has 5-8 erect racemes. Also in the region, but somewhat less spectacular, are *A. ciliaris, A. myriacantha,* and *A. speciosa*. On the road from Grahamstown eastward to King William's Town and East London *A. microcantha* and *A. myriacantha* have been found, along with *A. ecklonis* and other species.

The Transkei (recently declared an independent state) is a region to the northeast of East London and separated from it by the Great Kei River. It extends to Natal and is inhabited by the Xhosa tribe. Prominent aloes that have been studied from the Transkei are *A. arborescens, A. bainesii, A. boylei, A. ecklonis, A. ferox, A. kniphofioides, A. pratensis, A. saponaria* and var. *brachyphylla, A. tenuior* var. *rubriflora,* and *A. thraskii*. Just north of the eastern end of the Transkei is Griqualand East, inhabited by the Griquas, a race of mixed European and Hottentot origin. The Hottentots were a yellow race resembling Mongolians; they were one of the aboriginal races of the southernmost parts of South Africa but have almost completely died out. *A. aristata, A. boylei, A. ecklonis, A. kniphofioides,* and *A. pratensis* are found there and all have been reported from regions discussed previously.

North and east of the Transkei and Griqualand East is the province of Natal, where many aloes grow. One of the most interesting regions for succulents is the beautiful South Coast, which extends for a number of kilometers along the Indian Ocean. Along the coast, at Durban and at Pietermaritsburg, which is about 90 km inland, are *A. arborescens* var. *natalensis, A. mudenensis, A. pluridens, A. pratensis, A. pruinosa,* and *A. saponaria,* as well as several species that have not been studied cytologically, such as *A. candelabrum, A. kraussii, A. linearifolia, A. minima,* and *A. spectabilis*. In the northern part of Natal, around Dundee and Ladysmith, where the Second Anglo-Boer War started, and not far from Estcourt, near where Sir Winston Churchill was captured by Boer soldiers, are

A. gerstneri and *A. ecklonis*. Jacobsen (1954) lists *A. aristata* from Estcourt, but this species is not found in Reynolds (1950). In the Biggarsberg Mountains near Wasbank, south of Dundee, is *A. arborescens* var. *natalensis*. This plant is recognized as a good variety by Jacobsen (1954) but not by Reynolds (1950), who considers it to be typical of and therefore a synonym of *A. arborescens*. In Zululand in northeastern Natal are *A. arborescens, A. dewetii, A. marlothii, A. myriacantha, A. parvibracteata* and var. *zuluensis,* and *A. saundersiae*. *A. bainesii, A. boylei, A. kniphofioides, A. rupestris, A. suprafoliolata, A. thraskii,* and *A. vryheidensis* have also been recorded from Natal.

To the west of Natal is the mountainous country of Lesotho, formerly the British High Commission Territory of Basutoland. It is separated from the Republic politically but not botanically. A number of species found there are also in neighboring Natal. In Lesotho, aloes studied cytologically include *A. aristata, A. ecklonis, A. ferox, A. polyphylla, A. pratensis, A. saponaria, A. striatula,* and *A. zebrina*.

Swaziland, now a separate country but formerly one of the three British High Commission Territories, is an enclave in eastern South Africa, north of Natal. Among the aloes there are *A. arborescens* vars. *arborescens* and *natalensis, A. bainesii, A. boylei, A. chabaudii, A. chortolirioides* vars. *boastii* and *chortolirioides, A. cooperi, A. ecklonis, A. integra, A. kniphofioides, A. marlothii, A. parvibracteata, A. pretoriensis, A. rupestris,* and *A. suprafoliolata*.

In the northernmost part of the Republic is the Transvaal, a large region with many species of aloes. For convenience in locating these plants geographically the Transvaal may be divided arbitrarily into five regions. The southeastern part will be designated as the area east of the Great North Road between Vereeniging and Pretoria and south of a line east from Pretoria to Komatipoort. In this area are Belfast, Barberton, Ermelo, Piet Retief, Heidelberg, and Standerton. The east central region in this scheme is east of the road from Pretoria to Potgietiersrus and between the Pretoria-Komatipoort line and a line from Potgietersrus east to Mozambique. This region includes the southern part of the Kruger National Park, some of the low veld area just a little above sea level, and some of the high veld west of the escarpment including Lydenburg and much wild mountainous country. The southwestern area is west of the Vereeniging-Pretoria road and south of the road from Pretoria through Rustenburg to Zeerust; it includes Ventersdorp, Potchefstroom, Wolmaransstad, Bloemhof, and Schweizer Reneke. This is heavily settled country. North of this area to an arbitrary line west from Potgietersrus is a region designated here as the west central region; it is characterized by wild country and few people. Territory north of the arbitrary line running from Botswana to Mozambique through Potgietersrus can be regarded as the northern region. This is largely sparsely populated country but includes a few towns, such as Pietersburg, Louis Trichardt, and Messina.

Plants studied cytologically from the southeastern region are *A. albida, A. ammophila, A. bainesii, A. barber-*

toniae, A. boylei, A. burgersfortensis, A. castanea, A. chortolirioides, A. cooperi, A. dewetii, A. dyeri, A. ecklonis, A. graciliflora, A. integra, A. kniphofioides, A. longibracteata, A. marlothii and var. *bicolor, A. mutabilis, A. nubigena, A. parvibracteata, A. petricola, A. pretoriensis, A. reitzii, A. saponaria, A. simii, A. suprafoliolata, A. transvaalensis,* and *A. verecunda*. In the east central region are found *A. aculeata, A. ammophila, A. arborescens, A. boylei, A. burgersfortensis, A. castanea, A. chabaudii, A. cooperi, A. cryptopoda, A. davyana,* A. fosteri, A. globuligemma, A. integra, A. kniphofioides, A. longibracteata, A. marlothii, A. pretoriensis, A. reitzii, A. simii, A. verdoorniae,* and *A. verecunda*. *A. davyana* var. *subolifera* is near Pienaarsrivier. *A. davyana, A. grandidentata, A. mutabilis, A. transvaalensis,* and *A. verecunda* are in the southwestern part of the Transvaal. The first, *A. davyana,* is widespread and has been collected at Rustenburg and Zeerust. *A. grandidentata* has been collected at Bloemhof, Schweizer Reneke, and Wolmaransstad. *A. verecunda* is in the Johannesburg area. Few plants have been studied from the west central area; *A. davyana* var. *subolifera, A. mutabilis,* and *A. transvaalensis* have been found along the Great North Road. Aloes found in the northern Transvaal are *A. aculeata, A. angelica, A. arborescens, A. boylei, A. branddraaiensis, A. castanea, A. chabaudii, A. cryptopoda, A. fosteri, A. greatheadii, A. immaculata, A. lutescens, A. marlothii, A. mutabilis, A. pretoriensis, A. verecunda, A. vogtsii, A. vossii, A. wickensii* and var. *lutea*. *A. arborescens* and *A. verecunda* are found at Haenertsburg. *A. globuligemma* has been collected at Olifantsrivier in the Kruger National Park near Mozambique, and *A. arborescens,* a very widely disseminated species throughout South Africa, has been collected also in the Soutpansburg in the far northern part of the Transvaal near Rhodesia.

South of the Transvaal and west of Lesotho and northern Natal lies the Orange Free State. This whole country lies high on the large inland plateau and over 1,800 m above sea level on the average, with peaks going up to about 4,000 m. The country slopes gradually from the high Drakensberg range at the eastern side to a lower elevation on the western boundary. It is a prairie region of grassland with karroo bushes in the western part and no trees except those near streams and rivers or those planted by man. Unlike the Cape Province, most of which has a Mediterranean type of vegetation, the climate is continental with very hot summer days, cool summer nights, cool winter days, and very cold winter nights. The mean daily maximum temperature in the hottest month (January) is 30° C, and the mean daily minimum temperature in the coldest (July) is 0° C. Aloes found there and studied cytologically are *A. aristata, A. claviflora, A. davyana, A. ecklonis, A. ferox, A. grandidentata, A. hereroensis, A. ramosissima,* and *A. variegata*. Among those not studied are *A. areni-*

*Reynolds (1950) spells this species *Davyana* but Jacobsen (1954, 1960, 1970) prefers *Davyiana*. Schönland, who first described it (Rec. Alb. Mus. 1:288, 1903) lists it as "*A. Davyana*, Schönl., n. sp." This spelling is used in this book, but with a lower-case *D* to conform with modern rules.

cola, *A. broomii*, and *A. saponaria* var. *ficksburgensis*.

West of the Transvaal and the Free State is the northwestern part of the Cape Province, with Mafeking the most northerly city and Kimberley about 320 km to the south. Southwest of Kimberley is Hopetown and west of that is Prieska. North of Prieska is a vast, dry region that includes the town of Upington and the region of Griqualand West. It is an area of little rainfall and much sandy desert. In this region are *A. broomii*, *A. claviflora*, *A. grandidentata*, and *A. hereroensis*.

To the north of Little Namaqualand and the Richtersveld is South-West Africa. This large territory was once German South-West Africa and is now sometimes referred to as Namibia from the Namib Desert, which extends along the sandy coast. Species found there include *A. claviflora*, *A. dichotoma*, *A. grandidentata*, *A. hereroensis*, *A. karasbergensis*, *A. melanacantha*, a form of *A. microstigma*, *A. pearsonii*, *A. rubrolutea*, *A. variegata*, and *A. zebrina*. A number of other species inhabit this vast, dry area but have not yet interested cytologists or have not been available to them.

North of the northern Cape Province and west of the Transvaal is Botswana (formerly the Bechuanaland Protectorate), an extensive territory that includes the greater part of the Kalahari Desert. Aloes found there include *A. cryptopoda*, *A. globuligemma*, *A. grandidentata*, *A. greatheadii*, *A. littoralis*, *A. pretoriensis*, *A. rubrolutea*, *A. variegata*, and *A. zebrina*.

Angola (formerly Portuguese West Africa) lies north of South-West Africa. It rises gradually from the sea coast to an elevation of about 1,500 m. Aloes found there include *A. andongensis*, *A. buettneri*, *A. christianii*, *A. cryptopoda*, *A. hereroensis*, *A. littoralis*, *A. mzimbana*, *A. palmiformis*, and *A. zebrina*, several of which have been encountered in other regions previously discussed.

The large country of Rhodesia (formerly Southern Rhodesia, now sometimes called Zimbabwe) is located north of the Transvaal and Botswana and west of northern Mozambique. Found there are *A. arborescens*, *A. ballyi*, *A. cameronii*, *A. chabaudii* and var. *verekeri*, *A. christianii*, *A. cryptopoda*, *A. excelsa*, *A. globuligemma*, *A. greatheadii*, *A. hildebrandtii*, *A. littoralis*, *A. myriacantha*, *A. pretoriensis*, *A. saponaria*, and *A. zebrina*. Other species found there are *A. aculeata*, *A. chimanimaniensis* (named from the Chimanimani Mountains and considered a synonym of *A. swynnertonii* in Reynolds [1966]), *A. hazeliana*, *A. howmanii*, *A. inyangensis*, *A. munchii*, *A. musapana*, *A. ortholopha*, *A. plowesii*, *A. rhodesiana*, *A. swynnertonii*, and *A. wildii*, but no chromosome studies have been made from them.

Mozambique lies along the Indian Ocean from Tanzania to South Africa and on the west touches Malawi, Zambia, Rhodesia, the Transvaal, and Swaziland, and on the south Natal. It was formerly Portuguese East Africa. Its flora resembles that of the countries it touches. Species of *Aloe* found in Mozambique the chromosomes of which have been studied are *A. arborescens*, *A. bainesii*, *A. cameronii*, *A. chabaudii*, *A. christianii*, *A. cryptopoda*, *A. excelsa*, *A. greatheadii*, *A. littoralis*, *A. mawii*, *A. parvibracteata*, *A. rupestris*, *A. suffulta*, and *A. zebrina*.

The studies on Mozambique species have not been made from specimens from Mozambique but from those collected elsewhere. Other species have been found in Mozambique and a number of them are also in Rhodesia.

North of Rhodesia is Zambia (formerly Northern Rhodesia). Species recovered from there are *A. buettneri, A. cameronii, A. chabaudii, A. christianii, A. cryptopoda, A. greatheadii, A. littoralis, A. mzimbana, A. veseyi,* and *A. zebrina.* Many of the same species are found in Malawi, formerly Nyasaland, which is between Zambia and Mozambique. Malawi aloes include *A. arborescens, A. buettneri, A. cameronii, A. chabaudii, A. christianii, A. cryptopoda, A. excelsa, A. mawii, A. myriacantha,* and *A. mzimbana.*

East Africa consists of Kenya, Tanzania (formerly Tanganyika and Zanzibar), Uganda, Rwanda, and Burundi. A smaller percentage of the indigenous species has been studied here than in South Africa. Of those that have been studied, most of the chromosome determinations were made from South African rather than from East African material, but a few East African species not found in South Africa have been examined for their chromosome numbers and structure. The plants studied from this area include *A. amudatensis, A. ballyi, A. chabaudii, A. christianii, A. confusa, A. dawei, A. dorotheae, A. graminicola, A. lateritia, A. macrosiphon, A. mawii, A. myriacantha, A. mzimbana, A. ngobitensis,* and *A. rabaiensis.*

North of Kenya around the Horn of Africa are Ethiopia, Somalia, and the Sudan. A few species of *Aloe* that grow in this region have been studied. They are *A. eru* and its varieties, *A. jucunda, A. macrocarpa* and var. *major, A. megalacantha, A. percrassa, A. pirottae, A. rabaiensis, A. schweinfurthii, A. somaliensis, A. steudneri,* and *A. trichosantha. A. eru* and varieties are found in southern Egypt and *A. squarrosa* on the island of Socotra in the Indian Ocean near the Gulf of Aden.

Just north of Angola and Zambia and west of Tanzania is the large country of Zaire, formerly the Belgian Congo. Here are found several species that are also found to the south plus one or two others. They are *A. buettneri, A. chabaudii, A. christianii, A. dawei, A. greatheadii, A. lateritia, A. mzimbana,* and *A. schweinfurthii.*

A few species have been studied in West Africa. They are *A. buettneri* from Dahomey, Ghana, Mali, and Togo; *A. keayi* from Ghana; *A. macrocarpa* var. *major* from Dahomey and Nigeria; and *A. schweinfurthii* from Cameroons, Ghana, and Nigeria. *A. keayi* is generally believed to be a hybrid (Newton 1976). *A. buettneri, A. macrocarpa,* and *A. schweinfurthii* are usually considered to be natives of West Africa; Holland, however, suggests that they may have originated elsewhere during prehistoric times and been taken to West Africa by traders or migrating people.

A number of species of *Aloe* are found on the island of Madagascar but not elsewhere. Eight of them have been studied and their chromosome numbers are listed in Table 5.1. At least six species of *Aloe* grow in Arabia but apparently no chromosome studies have been made on Arabian plants. According to Jacobsen (1954) one species has been recorded from India but it is highly doubtful that it is indigenous there. In the Canary Islands, *A. barbadensis* Mill. (= *A. vera* L.) has been collected at Tenerife and

Garachio. This species grows in Barbados, Bermuda, and Jamaica but it is very likely that it was introduced into those countries. According to Reynolds (1966) it was originally described by Linnaeus as *A. perfoliata* (var.) *vera*. It deserves specific rank but as a species does not acquire its former varietal name. Its earliest specific epithet is *barbadensis*. Its origin is uncertain. Linnaeus considered it to have come from India, but it may have been introduced there from Africa. It has been cultivated (and escaped) since early times all around the Mediterranean shores and has been collected in the Canary Islands, the Cape Verde Islands, and the Island of Madeira. It is hard to conceive that it arose independently in the West Indies and other parts of the New World; it was probably introduced there during an early period of Spanish colonial history.

The preceding discussion of the aloes in Africa and elsewhere, although it deals almost exclusively with species that have been studied cytologically, indicates clearly that there is great variation in the distribution of the different species. For example, *Aloe buettneri* Bgr. extends from Angola, Zambia, and Malawi in its southern range northwards and westwards to Zaire, Mali, Nigeria, and Ghana. *A. myriacantha* (Haw.) R. et S. is found near Grahamstown in the Cape Province and in Kenya, Uganda, and Rwanda in East Africa, extending through Zululand, Rhodesia, and Tanzania in between. In southern Africa, *A. ferox* Mill. ranges from west of Swellendam to the Umtamvuna River (which separates the Transkei from Natal), and from Barrydale, northeast of Swellendam, to Aliwal North and to southern Lesotho and the Orange Free State. *A. marlothii* Bgr. extends from the Limpopo River and the eastern part of Botswana to the Klip River Hills south of Johannesburg and eastward and southward to Mozambique, Swaziland, and northern Natal. All four species cover extensive territories. On the other hand, *Aloe commixta* is restricted to the Cape Peninsula south of a line from Slangkop to Fish Hoek, while *A. umfoloziensis* is found only in some valleys in Zululand and in nearby Pongola. *Aloe arborescens, A. chabaudii, A. ecklonis, A. grandidentata, A. saponaria,* and *A. variegata* have been found in a large percentage of the regions mentioned above. The Aloineae in general and the genus *Aloe* in particular show great variation in their range and in the extent of the land they occupy.

Holland (1978) has pointed out that few *Aloe* species occur widely and that in *Aloe* the evolution of species has apparently been in the direction of filling a variety of niches. He has also shown that the first appearance of the ancestral aloes was probably in the southeastern corner of Africa during the late Mesozoic to early Tertiary era at a time when the island of Madagascar was still connected with the African mainland. This high region of southeastern Africa is probably the primary center of origin and the aloes probably migrated from there along the highlands of eastern and southern Africa as far as Arabia, where they became established near the end of the Tertiary. He suggests that from that primary center the aloes radiated out and that eleven secondary centers of speciation can be recognized.

Gasteria Duval

Gasteria, in comparison with *Aloe*, is a greatly restricted genus. *G. batesiana* has been recorded from Flentershoek in Zululand and in Piet Retief across the border from Zululand in the Transvaal; *G. croucheri* and *G. gracilis* are cited in Jacobsen (1954) as from Natal, but without a more specific designation. *G. transvaalensis*, as the name might indicate, is from the Transvaal; *G. ernestii-ruschii* seems to occur in South-West Africa at Lorelei on the Orange River a little above Sendelingsdrif. Except for these species, *Gasteria* seems to be limited to the Cape Province.

One of the regions where succulents are found in great numbers is the Little Karroo. In this area are *G. angustiarum, G. candicans, G. carinata, G. humilis, G. joubertii, G. loeriensis, G. obtusifolia, G. parvifolia, G. patentissima, G. schweickerdtiana, G. triebneriana*, and *G. vlaaktensis*. Some species are listed in some taxonomic monographs as being from the "Southern Cape Province," which may include the Little Karroo and more southerly regions. In this general area are found *G. angulata, G. angustifolia, G. bicolor, G. brevifolia, G. conspurcata, G. excavata, G. fasciata, G. glabra, G. laetipunctata, G. marmorata, G. mollis, G. picta, G. porphyrophylla, G. prolifera, G. spiralis*, and *G. sulcata*. *G. disticha* occurs in the Robertson Karroo and westward to Clanwilliam, and *G. maculata* is found along the coastal region near Outeniqua and Uitenhage. Along the southern area from west of Port Elizabeth to as far east as Uitenhage are *G. armstrongii, G. colubrina*, and *G. longibracteata*. Around Port Elizabeth and eastward to Grahamstown, King William's Town, and Algoa Bay are *G. acinacifolia, G. angustiarum, G. beckeri, G. chamaegigas, G. croucheri, G. fasciata* var. *polyspila, G. fuscopunctata, G. inexpecta, G. kirsteana, G. liliputana, G. longiana, G. lutzii, G. maculata* var. *dregeana, G. nigricans, G. nitida, G. pallescens, G. planifolia, G. salmdyckiana, G. stayneri, G. subverrucosa*, and *G. verrucosa*.

North of Port Elizabeth and Grahamstown and around Cradock are *G. armstrongii, G. biformis, G. caespitosa*, and *G. multiplex*. *G. variolosa* is found in the vicinity of Algoa Bay, Assegaibos to the west, and Cradock to the north. Southeast of Cradock toward Stutterheim is *G. huttoniae*. *G. neliana* and *G. pillansii* are in the Richtersveld, Little Namaqualand, and near Clanwilliam; *G. obtusa* is near Douglas, between Kimberley and Prieska. Some species are from areas that have not been clearly identified or they were studied from specimens in botanical gardens or from collections that did not identify the region of origin. They include *G. herreana, G. poellnitziana, G. pseudonigricans, G. pulchra, G. radulosa, G. retata, G. subcarinata, G. thunbergii, G. trigona*, and *G. zeyheri*.

Haworthia Duval

Haworthia is a larger genus than *Gasteria* in that it possesses more species, but it occupies the same general regions, being very abundant in the Little Karroo and in the

Port Elizabeth-Grahamstown areas. It is found almost exclusively in the Cape Province.

There are many species in the Worcester-Caledon-Montagu-Robertson-Touwsrivier area. This region includes the Robertson Karroo, often considered an extension of the Little Karroo. Probably restricted to this are are *H. angustifolia* vars. *denticulifera* and *subfalcata; H. arachnoidea; H. caespitosa* f. *caespitosa; H. emelyae* var. *beukmanii; H. guttata; H. hurlingii* and var. *ambigua; H. maraisii; H. margaritifera* var. *maxima* subv. *maxima; H. mclarenii; H. notabilis; H. pallida* and var. *paynii; H. reticulata* and var. *acuminata; H. schuldtiana* and vars. *erecta, minor, robertsonensis, subtuberculata,* and *whitesloaneana; H. setata* var. *nigricans; H. triebneriana* vars. *depauperata, diversicolor, nitida, pulchra, rubrodentata,* and *subtuberculata;* and *H. turgida* var. *pallidifolia.* In the same Robertson Karroo area but also to the south near Bredasdorp and Napier (or perhaps collected only in the latter region) are *H. aegrota; H. badia; H. mucronata* var. *bicarinata; H. mundula; H. mutabilis; H. nitidula; H. otzenii; H. rossouwii; H. schuldtiana* var. *maculata;* and *H. triebneriana* vars. *multituberculata, napierensis, sublineata,* and *turgida.*

To the east of the Robertson Karroo is the Little Karroo around Oudtshoorn, Ladismith, and Calitzdorp. In this area and/or the area to the south around Riversdale and Mossel Bay are many species. Some seem to be more or less restricted to this area or have so far been reported only from this region. They are *H. angustifolia* var. *liliputana; H. asperiuscula* var. *sub-integra; H. atrofusca; H. attenuata* var. *caespitosa; H. bilineata* var. *gracilidelineata; H. blackburniae; H. caespitosa* f. *subplana; H. chlorocantha* and var. *subglauca; H. correcta; H. cuspidata; H. cymbiformis* var. *translucens; H. dekenahii* and var. *argenteomaculosa; H. ferox* var. *armata; H. floribunda; H. fouchei; H. graminifolia; H. habdomadis; H. heidelbergensis; H. integra; H. laetevirens; H. lateganae; H. longebracteata; H. marginata; H. marumiana; H. maughanii; H. monticola; H. morrisiae; H. mucronata* var. *limpida* f. *inconfluens* and f. *limpida; H. mucronata* var. *morrisiae; H. nigra* var. *angustata; H. papillosa; H. paradoxa; H. parksiana; H. picta; H. planifolia* var. *transiens; H. pseudogranulata; H. pygmaea; H. retusa* vars. *densiflora, multilineata,* and *solitaria; H. schuldtiana* and vars. *major* and *simplicior; H. semiglabrata; H. setata* vars. *gigas* and *joubertii; H. smithii; H. starkiana, H. sublimpidula; H. tenera* var. *major; H. tessellata* var. *tuberculata; H. tisleyi; H. triebneriana* var. *lanceolata; H. truncata* and formae *crassa, normalis,* and *tenuis; H. tuberculata* and vars. *acuminata, angustata, subexpansa,* and *sublaevis; H. turgida* var. *suberecta;* and *H. viscosa* vars. *caespitosa* and *subobtusa.* Some species and varieties are found in the Robertson Karroo-Bredasdorp general area and the Little Karroo. They include *H. aristata, H. asperula, H. haageana, H. magnifica, H. obtusa* var. *columnaris, H. pallida, H. submaculata, H. turgida, H. variegata, H. venteri,* and *H. viscosa* var. *indurata.*

To the east of the Little Karroo is a fabulous region for observing succulent plants, primarily the flat Great Fish River Valley. In this area the rainfall, which comes

mostly in the summer months, is only about 32-50 cm a year. The elevation is generally from 90 to 450 m. In its undamaged state the veld consists of very dense, semi-succulent, thorny scrub. Grazing (and sometimes over-grazing) has opened up much of the region; and it has been invaded by *Euphorbia bothae* and the American prickly pear, which is considered by South African botanists to be a menace. With the Fish River Valley will be included here all the region from Port Elizabeth and Uitenhage to the Stockenstrom Division, near Seymour, and through the Transkei to the Natal border.

Found here are *H. angustifolia* var. *grandis; H. armstrongii; H. attenuata* var. *clariperla; H. baccata; H. browniana; H. carrissoi, H. cymbiformis* vars. *angustata, brevifolia, compacta, multifolia,* and *obesa; H. fasciata* and formae *major, ovato-lanceolata, sparsa, subconfluens, vanstaadensis,* and *variabilis; H. fulva; H. greenii* and var. *silvicola* and f. *pseudocoarctata; H. haageana* var. *subreticulata; H. incurvula; H. intermedia; H. isabellae; H. leightoniae; H. lepida; H. longiana* and var. *albinota; H. luteorosea; H. margaritifera* var. *minima* subv. *polyphylla; H. mucronata* var. *limpida* and f. *acuminata; H. mucronata* var. *polyphylla* and f. *minor* of that variety; *H. mucronata* var. *setulifera; H. musculina; H. nigra* and vars. *elongata* and *suberecta; H. obtusa* var. *dielsiana* and f. *acuminata; H. obtusa* vars. *gordoniana, salina,* and *stayneri; H. perplexa; H. planifolia* var. *longifolia* f. *calochlora,* and vars. *setulifera* and *sublaevis,* and var. *planifolia* formae *agavoides, alta, olivacea,* and *robusta; H. radula; H. ramosa; H. reinwardtii* and at least 18 of its named varieties; *H. reticulata; H. rugosa; H. setata* var. *xyphiophylla; H. sordida; H. stiemei; H. subfasciata; H. tenera; H. tessellata* var. *minutissima; H. umbraticola; H. viscosa* var. *quaggaensis;* and *H. vittata. H. reinwardtii* is a species with a large number of named varieties. The differences among them are small, and they are difficult to identify. Succulent fanciers like them but the local botanists in the area generally seem to be disinterested in identifying to the variety wild plants of this species.

In both the Little Karroo and southern areas and the Great Fish River-Transkei areas are *H. altilinea* var. *denticulata, H. mucronata, H. recurva,* and *H. subulata. H. angustifolia* var. *albanensis, H. planifolia* var. *planifolia,* and *H. translucens* are in these regions plus the Bredasdorp area. Several species are in the Great Karroo: *H. asperiuscula* var. *patagiata; H. attenuata* var. *attenuata; H. batesiana; H. bolusii* vars. *aranea* and *semiviva; H. comptoniana; H. decipiens; H. eilyae* var. *eilyae; H. helmae; H. herrei* var. *herrei; H. jacobseniana; H. jonesiae; H. margaritifera* var. *corallina; H. nigra* var. *diversifolia* f. *nana* and var. *pusilla; H. planifolia* var. *incrassata; H. reinwardtii* var. *chalwinii; H. tenera* var. *confusa; H. triebneriana; H. viscosa* vars. *cougaensis, pseudotortuosa,* and *viridissima; H. willowmorensis; H. wittebergensis;* and *H. woolleyii.*

Found in the Great Karroo and in one or more of the adjoining areas are *H. altilinea* var. *altilinea, H. angustifolia, H. asperiuscula, H. attenuata* var. *britteniana* f. *britteniana, H. bolusii, H. caespitosa, H. coarctata, H.*

cooperi, H. ferox, H. gracilis, H. herrei var. *depauperata, H. margaritifera, H. mucronata* var. *limpida* f. *inermis, H. nigra* var. *schmidtiana, H. obtusa* and var. *pilifera, H. retusa* and var. *mutica, H. tessellata* and var. *inflexa, H. turgida* var. *subtuberculata, H. venosa,* and *H. viscosa* and var. *torquata*. East of the Great Karroo near Queenstown or in that area and the Great Karroo are *H. blackbeardiana* and var. *major, H. tessellata* var. *parva,* and *H. zantneriana*.

A few species or varieties such as *H. caespitosa* f. *subproliferans, H. globosiflora, H. granulata, H. mucronata* var. *morrisiae* f. *subglauca, H. nortieri* and vars. *giftbergensis* and *montana, H. setata* var. *bijliana,* and *H. uitewaaliana* are found in the northwest corner of the Cape Province around Little Namaqualand, the Richtersveld, Calvinia, Clanwilliam, and the Roggeveld Mountains. Farther east near Prieska are *H. nigra* var. *diversifolia* and *H. viscosa*. *H. tessellata* vars. *elongata, engleri,* and *tessellata* are found in the southern part of South-West Africa.

Some taxa have been named and described from cultivated specimens and some without any given locality except sometimes a vague one, such as the Cape Province. Among them are *H. bilineata; H. herbacea; H. nitidula* var. *opaca; H. peacockii; H. pearsonii; H. planifolia* vars. *exulata* and *poellnitziana; H. reinwardtii* vars. *minor, olivacea,* and *triebneri; H. resendeana; H. revendettii; H. rigida* and var. *expansa; H. rubrobrunea; H. ryderiana; H. sampaiana* and f. *broteriana; H. schuldtiana* var. *sublaevis; H. sessiliflora; H. setata* vars. *major* and *media; H. sordida* var. *agavoides; H. subattenuata; H. subfasciata* var. *kingiana; H. subregularis; H. tessellata* vars. *coriacea, luisieri, obesa, stephaneana,* and *velutina; H. tortuosa* and vars. *major, pseudorigida,* and *tortella; H. translucens* var. *delicatula; H. umbraticola* var. *hilliana;* and *H. viscosa* var. *concinna*. According to Jacobsen (1954), *H. tessellata* vars. *palhinhae* and *simplex* were named from plants growing in the botanical garden in Lisbon, Portugal, and *H. tortuosa* var. *curta* is a cultivated variety. Such taxa may be beautiful and valuable as ornamental plants but are of little use to a study of evolution in the tribe.

According to Jacobsen (1954), *H. glauca* is found in the Orange Free State; Bayer maintains that it is in the Zuurberg Mountains east of the Sundays River and northeast of Uitenhage, not in the Free State. Jacobsen also lists *H. limifolia* as found in Barberton in the eastern Transvaal and in the "Cape-Province: Gegend westlich der Delagra Bay." The actual location of this species is puzzling. Delagra Bay is obviously a misspelling for Delagoa Bay, and Rowley (Jacobsen 1960) has made the correction in his translation of Jacobsen's book. However, it is not in the Cape Province but is at Maputo (formerly Lourenço Marques) in Mozambique. According to Jacobsen the variety is found in the region or country to the west of Delagoa Bay. Depending on how far west one goes, this statement means in either Mozambique or the Transvaal. At the latitude of Delagoa Bay, Mozambique is only about 65 km wide, so the reference could easily be to the Transvaal. Barberton is only 160 km from Delagoa Bay, and this variety

probably is restricted to the Transvaal. In Jacobsen (1954) there are five other varieties of this species, one with three forms. Three of the varieties (including the one with three forms) are not located geographically, but *H. limifolia* var. *keithii* and *ubomboensis* have been found in the Ubombo Range in Swaziland. This region is very near Barberton but not near the Cape Province. Another indication that *H. limifolia* var. *limifolia* is in the Transvaal rather than Mozambique is that the species seems to be one of high elevation. Two species are known to be mountainous; although Barberton is in a valley, it is considerably higher than the Mozambique low veld and is surrounded by mountains. It is not too likely that a species would be partly in the high country west of the escarpment and partly in the region east of the escarpment, which rises little above sea level.

Astroloba Uitew.

Astroloba Uitew. (= *Apicra* Haw.) is a small genus of 12 named species, several of which have two or more varieties. It is found chiefly in the southern part of the Cape Province. *As. bicarinata* is at Graaff-Reinet at the edge of the Great Karroo; *As. egregia* has been found in the Little Karroo at Oudtshoorn; and *As. herrei* is at Uniondale. *As. deltoidea* var. *turgida* is in the Albany Division, near Grahamstown. *As. deltoidea* var. *deltoidea* is in the adjacent Alexandria Division but is also found in the nearby Suurberg and in the Great Karroo at Laingsburg and Matjesfontein. *As. foliolosa* occurs between the Swartkops River and the Sundays River, probably in the Uitenhage Division; *As. aspera* is in the southeastern Cape Province at Springbokkeel. *As. bullulata; As. congesta; As. pentagona* vars. *pentagona, spiralis,* and *spirella; As. skinneri;* and *As. spiralis* are listed from the southern Cape Province. *As. deltoidea* var. *intermedia* and *As. dodsoniana* are listed simply from the Cape Province; *As. aspera* var. *major, As. egregia* var. *fardeniana,* and *As. pentagona* var. *torulosa* have no geographical designation in Jacobsen (1954).

Chamaealoe Bgr.

This is a monotypic genus closely related to *Aloe*. *C. africana* occurs in the southern part of the Cape Province.

Poellnitzia Uitew.

Poellnitzia is a monotypic genus. *P. rubriflora* var. *jacobseniana* has been found in the Cape Province at Worcester, and *P. rubriflora* var. *rubriflora* is listed only as from the southern Cape Province.

Chortolirion Bgr.

Five species of *Chortolirion* are described in Jacobsen (1954). *C. stenophyllum* and *C. subspicatum* are from the

Transvaal, the former at Johannesburg and Barberton and the latter from Modderfontein and near "Nummejaarsprint" (apparently a misprint in Jacobsen [1954] for Nuwejaar Spruit) near the Orange River at Aliwal North. *C. tenuifolium* is possibly located in the northern part of the Cape Province at Kuruman. The exact location of this species is not clear. According to Jacobsen it is in "Südl. Bechuanaland: Kumuran; Manjering bei Kubuman." Rowley's translation repeats this location. However, we cannot find any of these three places in the section on Bechuanaland in the 1960 *Post Offices in the Union of South Africa and Neighbouring Territories* published by the Government Printer in Pretoria. Could Kumuran and Kubuman be misspellings for Kuruman, a fairly well-known city in the Cape Province near Botswana? On the Kimberley SE 29/22 topographical map printed in 1963 by the Government Printer, Pretoria, there is a Maneering not far from Kuruman. Is this the place indicated by Jacobsen and Rowley as Manjering? If so, this species is in the Cape Province, not Botswana. In Jacobsen (1970) its location is given only as "S-Botswana." Two species are beyond the borders of South Africa: *C. bergerianum* is in South-West Africa, and *C. angolense* is in Angola.

Lomatophyllum Willd.

Twelve species are listed, none of which occurs in South Africa, or on the mainland of Africa, for that matter. Nine are on the island of Madagascar, one is on the island of Mauritius, one is on both islands, and one is on both and also on the island of Aldabra.

Leptaloe (Stapf) Bgr.

This genus is often included as a section of *Aloe* L. Eighteen species are in South Africa (near Grahamstown, in Natal, and in the eastern Transvaal). Eleven are in tropical Africa, chiefly in Rhodesia, Zambia, Malawi, Tanzania, Kenya, Uganda, and Angola.

Guillauminia A. Bertr.

Gu. albiflora (Guill.) Bertr. (= *Aloe albiflora* Guill.) is found only in southern Madagascar.

Chapter Three

MATERIALS AND METHODS FOR STUDY

Any study dealing with evolutionary problems in plants must consider the sources of the materials used — i.e., the places where and the conditions under which the plants forming the study were obtained. In considering the Aloineae two main sources of material have been used: collections growing in botanical gardens and collections made from the veld or natural regions where the plants are growing.

With plants collected directly from the veld there are no particular problems of authenticity. Such plants are the end products of evolution to date and the raw materials of future evolution. They show the exact state of evolution of varieties, species, and genera at the moment of study and indicate trends which may possibly be used to interpret future evolutionary changes. No one could criticize the use of wild plants in the interpretation of evolution in a given taxon.

Plants found in collections of botanical gardens or of succulent fanciers, however, are less reliable, generally in proportion to the time they have existed in the collection. They are subject to various sources of error. For example, large collections in botanical gardens are frequently watered and otherwise cared for by people who are excellent gardeners but are not trained scientifically to appreciate the significance of pot labels. Occasionally the labels are allowed to become illegible and sometimes even lost. Labels that are lost are sometimes replaced but not always in the correct pot; incorrectly placed labels are even worse than lost labels. The confusion of pot labels has occurred occasionally even in well-known collections, and the older the collection the more likely that it has happened. Furthermore, a collection might have been assembled by a botanist who was a serious student of succulents, but upon his retirement no one else at his institution was interested in his collection. It might stay around for several decades in a state of semi-neglect simply because of a loss of interest in that group of plants. A generation or two later another botanist may appear at the institution who has an interest in the group and who proceeds to study it. How has the collection changed during that period of neglect? It is here that the problem of labels is so compelling. In addition, mu-

tations may have arisen that over a long period of time make some of the specimens unrecognizable. In clonal plants, as most of the Aloineae are, a side shoot may appear that bears a somatic mutation, and it may grow and develop. If the original shoots die, or if a gardener cuts off and pots this side shoot separately with a duplicate of the original label, confusion will result. Also the taxonomy of a succulent group is rather specialized, and few taxonomists are primarily interested in succulent plants.

Sources of Materials

Cytogeneticists who have studied the chromosomes of the Aloineae have obtained their material from various sources. The earliest studies apparently were made on a few plants that happened to be lying around in a greenhouse for a number of years and did not form any part of a collection of the Aloineae or even of succulents in general and, so far as one can tell from the published accounts, whose original habitat was not recorded. In their early work neither Clemens Müller (1912) nor W. R. Taylor (1924, 1925a, b, c) mentioned the source of his material, which comprised *Aloe hanburyana* Naud. (= *A. striata* Haw.) for the first author and *Aloe arborescens* Mill., *A. saponaria* Haw., *Gasteria verrucosa* (Mill.) Haw., *G. cheilophylla* Bak., and *Haworthia cymbiformis* Haw. (var. *obtusa* Bak.?) for the second. Ferguson (1926) also worked with plants that apparently had been in a botanical garden for some time. Her work was much more extensive than that of her two predecessors, and her material came chiefly from a collection of succulent plants growing in the Royal Botanical Gardens at Kew. She also studied a group of species of *Gasteria* and *Haworthia* obtained from an "interesting collection of plants belonging to W. Horton, Esq., of Liverpool." How long these plants had been away from their native habitats was not stated, and if there were any records of the natural regions from which the plants had been collected they were not available. Evolutionary data from such collections are entirely unreliable.

A decade later Sato (1937) published an extensive list of chromosome numbers in *Aloe, Gasteria,* and *Haworthia*. The materials for this study were all from potted plants, most of which had been raised from seeds imported by the Koisikawa Botanical Garden of Tokyo Imperial University. He stated: "For the species name the label of the imported seed bag was adopted in most cases." His material is subject to all the usual sources of error inherent in any botanical garden collection of plants and also to the problem of the constancy of seeds. There is considerable interspecific and even intergeneric hybridization in the Aloineae, and to trust the seeds from homozygous plants to be true is too naive. The seeds he used were very possibly genetic segregates from highly heterozygous parents; this situation would reduce to almost nothing the value of his lists of chromosome numbers. Kondo and Megata (1943) also published a list of chromosome numbers in these three genera plus one species of *Astroloba* and included some interesting figures of chromosomes from meiotic and micro-

spore divisions. However, their paper is written in Japanese, which neither of the present authors can read. Their English résumé lists the chromosome numbers (*n* or 2*n* or both) in 37 taxa but makes no mention of the source of the material.

A few people have reported on just one or a few plants from botanical garden sources. Giles (1943) described an interesting plant with an isochromosome. It was a species of *Gasteria,* probably *G. maculata,* and was from a collection of miscellaneous plants in the botany department of Yale University. Its original habitat was unknown. Vig (1968) studied chromosomes of a plant of *Aloe barbadensis* Mill. (as *A. vera* L.) and stated that the plant was "of an unidentifiable (and perhaps some indigenous) variety"; but he does not state where he obtained it or where it was indigenous. Surely not in Columbus, Ohio, where he was working! A somewhat larger collection of plants was covered by Marshak (1934), who studied three species of *Aloe,* twelve of *Gasteria,* and three of *Haworthia.* Unfortunately no mention was made of the origin of the plants; presumably they were found in one of the botanical gardens of Harvard University where the work was done, and presumably they had been growing for a long time far from their native Africa. As that author wisely stated, "One hesitates to draw inferences about wild species from representatives grown long under cultivation."

Two series of studies on better authenticated material are those of Müller (1941, 1945) in Pretoria and Snoad (1951a) and Rowley (1967) in England. Müller's work was confined to the genus *Aloe.* His plants were largely from the National Herbarium in Pretoria, but some were from botanists in Pretoria and Johannesburg. Since many of his identifications were made by Reynolds, they must be regarded as being as authentic as any can be. In describing his materials and methods, Müller stated that his study was based on materials identical with the "type plant," either being collected from the type locality or being a well-represented example.

Snoad's work was based on a collection of succulent plants established at the John Innes Institute at Norwich (formerly the John Innes Horticultural Institution) by G. D. Rowley, who helped with the identification of certain species. Rowley's collection was in part received from J. T. Bates of Hounslow, England. Rowley stated that it is usually well documented, having been received direct from collectors and botanists and carefully recorded in diaries. His *Gasteria batesiana* was received in 1942 from Kew, but its South African origin is unknown. These serious collections of succulents made quite recently should be much more authentic than plants of the Aloineae found in a general collection of miscellaneous families made many years ago. Brandham's studies are more recent. He worked with 177 plants which were part of a current collection at the Royal Botanic Gardens, Kew. He made a thorough attempt to verify the identity of the plants by checking the descriptions in Jacobsen and Reynolds. A few plants could not be identified. In a 1971 paper Brandham mentions that many of the *Aloe* species were identified by P. R. O. Bally or G. W. Reynolds.

One of the most extensive chromosomal studies of the

Aloineae was made in Portugal by Flavio Resende and his associates. Their studies were made on a large collection of succulents called "the Resende collection." His plants came largely from the Hamburg Botanic Garden; a few were from the Berlin and Breslau gardens and from Blankenese, and a few were from Coimbra. As he stated (Resende 1936), most of his species were cultivated in the Hamburg Botanic Garden. For example, his *Aloe mitriformis* var. *commelinii* Bak. was a greenhouse plant growing in the Hamburg Botanic Garden (Resende 1940b), and his early study of *A. ciliaris* was from two cuttings found in a pot; one was a *gigas* form. In his 1943 paper Resende stated that his plants of the Aloineae were imported from Africa and cultivated in the botanic garden in Portugal. Habitats were listed sometimes but not always, and there was no mention of when the plants were removed from their habitats. Many of the habitats listed were vague and general rather than specific for the individual specimens collected; for *H. reinwardtii* and some of its varieties Resende (1943) gave the location as the southeastern Cape Province and mentioned that von Poellnitz considered Alicedale as the center of the area. Pinto-Lopes (1946) stated that the specimens were "potted and maintained in the green-house or in the open in the Botanical Garden at Lisbon." Resende (1948) stated that he started with the greater part of the living types of von Poellnitz belonging to the Coarctatae section in 1937. These identifications should have been reliable. On the other hand, Viveiros's study (1959) comprised 356 plants of the Coarctatae section that had been raised from seeds. The origin of the seeds was not given; even had it been, it would have been unimportant since cytotaxonomic studies based on seeds are unreliable.

In Appendix i of Bayer's (1976) *Haworthia Handbook,* Resende's taxonomic work is reviewed. Bayer believes that only a few of Resende's names can be taken seriously and considers that not one is now upheld as a specific name. One of his chief criticisms is that nearly all Resende's plants were of unknown or garden origin and his collection included polyploid plants that were hybrids.

Newton (1970) listed chromosome numbers for four species of *Aloe* from tropical western Africa and a variety of one of them from Uganda. They were studied very soon after they were collected, and the country and the part of the country where they were collected were listed. For some of the species several clones were obtained — an important feature for biosystematic studies.

The work of Sharma and Mallick (1965) is quite different from Newton's. They studied 40 species of *Aloe, Gasteria,* and *Haworthia* and reported that a large number of individuals of each species were examined but evidently all the plants of a given species belonged to a single clone. Their plants were assembled from Imperial Nursery at Calcutta, Chandra Nursery at Kalimpong, and Ghosh's Nursery at Darjeeling, and also from private gardens. There was no indication of the habitat of any of the species, of the persons who collected them in their native countries and sent them to India, or of the approximate length of time they had been growing in the Indian nurseries. Presumably the nurseries were commercial. Sharma and Mallick stated that the species "have been properly

identified and verified from the Indian Botanical Gardens of Shibpur," but one might well wonder whether Sharma and Mallick appreciated the difficulty of identification of the Aloineae by anyone not a specialist in the group. They further stated that "some of them are horticultural." Horticultural plants are often interesting with many problems of their own but should not be used for biosystematic or evolutionary studies.

The plants of the present writers and their associates were obtained from a number of different sources. The first plant studied, a *Gasteria sulcata* X *G. nigricans* triploid, had been received in 1938 from the Huntington Botanic Gardens at San Marino, California; two *Gasteraloe* hybrids studied at about the same time came from Charles Cass of Pacific Grove, California. Of the early work on *Gasteria* most of the plants came from the National Botanic Gardens of South Africa through the courtesy of the director, Professor R. H. Compton. They were studied chiefly from 1947 to 1952. The senior author (Riley 1959b) also published a study on *Gasteria* which included some plants he collected in the field personally. Fifty plants of *G. zeyheri*(?) were from the Bushman's River Poort and eight of *G. beckeri* were growing on the bank of the nearby Kamtra River. In both species apparently no two individuals were from the same clone. Seven plants of *Haworthia reinwardtii* or its varieties were from the Kamtra River, and a plant of an unidentified *Astroloba* species was growing at the Great Fish River. Some plants were received from A. Berg of the University of Pretoria and Hans Herre of the University of Stellenbosch. All their identifications are much more reliable than those of plants from a botanical garden collection on another continent. In another study (Riley 1959a) other plants of the genus *Aloe* included 40 plants of *A. davyana* from De Wildt in the Transvaal; one of *A. ferox* from the Kamtra River; and plants of *A. speciosa* from the Great Fish River, *A. ciliaris* from the De Bega Valley, *A. mitriformis* from the Franschhoek Pass, *A. claviflora* from Professor E.A.C.L.E. Schelpe of the Bolus Herbarium, *A. striatula* and *A. rupestris* from the University of Pretoria Botanic Garden; 11 plants from A. Berg; and seeds from Harry Hall of Kirstenbosch. These studies represent the first chromosome studies based on natural populations. Nearly all the material published by the present authors during the past decade was of the genus *Haworthia* and was received from J. W. Dodson of Millbrae and later of Orindo, California. He has a marvelous collection known as the International Succulent Institute; he is an authority on the taxonomy of the group, has named some species, and has had some named for him. He obtained the plants direct by air from botanists in South Africa. It was through his kindness that the authors had so much *Haworthia* material for study, and his collection is far more reliable taxonomically than most other succulent collections in botanical gardens.

Herbarium Specimens

One of the severe criticisms (perfectly justifiable) of the early work in plant cytogenetics in the 1930s was the

failure of the cytogeneticists to document their material with herbarium specimens. This criticism was not so meaningful for studies of chromosome structure or chromosome behavior in which the identification of the species studied was not very important, but when cytogenetics developed into cytotaxonomy and then into biosystematics identification became crucial. The early cytogeneticists generally failed to appreciate the significance of documented studies, so many of the determinations of chromosome numbers made during that period are of doubtful value and should be disregarded. This statement is as true of the early workers in the Aloineae as it is of their contemporaries working in other genera. No references to the preparation of herbarium specimens can be found in the papers of Ferguson, Taylor, Heitz, Resende, Tuan, Marshak, Suto, Sato, Schnarf and Wunderlich, Müller, Kondo and Megata, Giles, Snoad, Rowley, Darlington and Kefallinou, Schelpe, Brinckmann, Sharma and Mallick, or Vig. Several people justify the lack of herbarium specimens by stating that their plants are in a permanent collection; Resende adds that herbarium specimens of the Aloineae are very poor, which is certainly true. However, one wonders how permanent a "permanent collection" can be and whether future successors in Resende's and others' positions may not decide they have better use for the space occupied by the collections. Müller's chromosome studies were made largely from a collection of aloes growing in the garden of the National Herbarium in Pretoria. Presumably the National Herbarium has filed herbarium specimens of his material, but he does not cite any voucher numbers. Brandham (1971) states that voucher specimens of his material are in the course of preparation.

The importance of herbarium specimens was well recognized by L. E. Newton (1970), who states: "Voucher specimens with the writer's collection numbers (Table 1) are in Kumasi University Herbarium..., Ghana, and clonal replicates are to be deposited in Kew Herbarium..., England, and Legon University Herbarium..., Ghana."

The importance of voucher specimens has been recognized by the present authors, who have pointed out the need for them in several papers. However, until 1974 we made no herbarium specimens because we felt that the living plants should be available in case further studies on them would be needed. From 1942, when this work was started, until 1974, when the senior author retired, occasional plants died and some others disappeared from the greenhouse because they are attractive as house plants. Voucher specimens of those that survived have been deposited in the Compton Herbarium of the National Botanical Gardens of South Africa at Kirstenbosch. Bayer (1976) has noted the filing of these specimens in his *Haworthia Handbook*.

Cytological Techniques

A study of the history of cytological methods used with the Aloineae very closely parallels the history of cytological methods used with plants in general. The first workers (H.A.C. Müller 1912; Ferguson 1926) employed the

paraffin method. Müller used root tips that were fixed, sectioned, and stained with Heidenhain's iron haematoxylin, aniline-eosin, and acid fuchsin-malachite green — a rather common method at that time. Ferguson studied pollen mother cells primarily and used "the usual cytological fixatives." Her best results generally came from standard and medium strengths of chromacetic acid. Allen's modification of Bouin's fixative (a fairly common fixative for animal tissues in the 1920s) was excellent for some species of plants but worthless for others. Chromosome shape was studied from somatic divisions of tapetal nuclei or from somatic metaphases in flower buds.

Taylor (1924) used paraffin sections only to study vegetative cells or to check on other methods. He chiefly studied developing microspores, which were squashed on the slide and fixed, stained, and mounted. Fixation was usually with a chromosome-fixing solution, such as Bouin's picro-formolacetic acid with chromic acid. Tuan (1930, 1931), working in Taylor's laboratory, used essentially the same methods. He smeared pollen mother cells, stained with haematoxylin and safranin, and differentiated with picric acid.

Heitz (1931) studied nucleoli primarily but also chromosomes of root tips, and he gave very detailed instructions. For telophase and interphase cells he preferred osmic acid containing little or no acetic acid, and found that the fixatives of Flemming, Benda, and Champy gave good results. (These were usually zoological fixatives.)

Sato's extensive list of chromosome numbers was compiled from sectioned material of root tips. Müller (1941) used Belling's iron-acetocarmine smear method in modified form for his early studies on *Aloe* and also made microtome sections, staining with Newton's gentian violet—iodine method. He thought both furnished good results, especially the squash method (smear method) of Belling, which he considered essential for determining chromosome numbers ("wat onmisbaar is as die aantal gromosome vasgestel moet word"). Later Müller (1945) used root-tip squashes. Resende and his group generally used squash methods and frequently referred to the "nukleal-Quetschmethode" of Heitz (1935).

In his recent extensive studies on the Aloineae, Brandham used essentially the same methods. For stages of meiosis (Brandham 1969a) he squashed fresh microsporocytes in 2% aceto-orcein, a stain that had replaced acetocarmine in studies of some plants for a number of years. Root tips received a different treatment. They were pretreated for 4½ hours in 0.002 M 8-hydrozyquinoline at 15° C, or in saturated alpha-bromonaphthalene overnight at 2° C (Brandham 1971) or for 24 hours at 4° C (Brandham 1975). They were then fixed in 1:3 acetic alcohol, hydrolyzed in 1N hydrochloric acid at 60° C for 6 minutes, and stained with the Feulgen technique. For anaphase and telophase stages of mitosis the pretreatment was omitted (Brandham 1975). The slides were made permanent by the liquid carbon dioxide method of Bowen (1956), which is much like the method Conger and Fairchild (1953) developed at Oak Ridge at about the same time. It is highly desirable that slides be rendered permanent so they can be studied by other cytologists, but the establishment of such a collec-

tion does not guarantee it will always be available; subsequent administrators might not recognize its research value.

Darlington and Kefallinou (1957) used acetocarmine squashes for meiosis and Feulgen squashes for mitosis. The present writers have frequently used colchicine or paradichlorobenzene to shorten the chromosomes, spread them apart from one another, and spread out the chromatid arms; other chemical compounds have been used by other cytologists for these and related purposes. Snoad (1951a) pretreated his root tips with alpha-bromonaphthalene, and Vig (1968) used a 0.02% solution of 8-hydroxyquinoline. Newton (1970) also preceded his root-tip squashes with immersion in 8-hydroxyquinoline.

Sharma and Mallick (1965) used several chemical compounds before they fixed their root tips. Among them were aesculin, oxyquinoline, and paradichlorobenzene; different concentrations were used at different times, and the last gave the best results. The material was pretreated for 10 minutes at 2-4° C, then for 3 hours at 18-20° C; it was fixed for ½ hour in a 1:2 solution of acetic acid and alcohol, heated gently for 5-6 seconds in a 9:1 mixture of 2% aceto-orcein and 1N hydrochloric acid, and then kept in that mixture for an hour and squashed in 1% aceto-orcein. Permanent preparations were fixed in Levitsky's fixative (equal parts of 1% chromic acid and 10% formalin) to which 0.002 M oxyquinoline was added in a ratio of three parts fixative and one part oxyquinoline. Temporary meiotic slides were made with acetocarmine and permanent slides by fixing in Navashin's solution and staining in Newton's crystal violet—iodine stain.

The present authors have generally used acetocarmine squashes for anthers and Feulgen squashes for root tips. Two methods have been used for the root tips: (1) pretreatment with alpha-bromonaphthalene and fixation in a mixture of osmic acid, chromic acid, and 8-oxyquinoline (Riley 1959a); (2) pretreatment in 0.1% colchicine, a saturated solution of paradichlorobenzene, and a saturated solution of aesculin mixed in a 2:1:1 solution, followed by fixation in a mixture of equal parts of 95% ethanol and glacial acetic acid (Riley and Mukerjee 1962). More recent studies by the present authors employed essentially the method Majumdar (1964a) developed for *Hevea* except that (1) the root tips after Feulgen staining were mounted in aceto-orcein instead of in 45% acetic acid and (2) to improve the spreading of the chromosome the root tips were pretreated with a saturated solution of paradichlorobenzene, aesculin, or a mixture of paradichlorobenzene and oxyquinoline.

The present writers' most widely used method is as follows:

1. Cut the root about 1 cm from the end and place for 3 hr in a sat. sq. sol of paradichlorobenzene at 10-12° C.
2. Fix the root tips for 24 hr in a 3:1 sol of 95% ethanol and glacial acetic acid.
3. Hydrolyze for 15 min in 1N HCl at 60° C.
4. Stain in Feulgen sol at 10-12° C until the meristematic region of the root tips is brightly stained.
5. Cut off the meristematic region in 1% aceto-orcein.

6. Seal coverslips in paraffin for temporary study.
7. To make permanent, invert slide in a 1:1 sol of 95% ethanol and glacial acetic acid, place 2-3 min in 95% ethanol, and mount in euparal.

Several recent cytological methods connected with cytochemical studies rather than cytogenetic studies are of passing interest and might appropriately be mentioned here; since they are not of primary interest to the biosystematist or cytogeneticist, full bibliographic references for them will not be included.

For differential staining of RNA and DNA, toluidine blue was suggested by Stevens in 1961, azure blue by Flax and Himes in 1952, and pyronin–methyl green by Bhaduri and Mukherjee in 1961. Mercury bromphenol blue was used by Bonhag in 1955 for total proteins, and alkaline fast green (Alfert and Geschwind in 1953) and eosin Y and bromphenol blue (Bloch and Hew in 1960) were used for histones.

For a study of the ultrastructure of the wall of *Haworthia, Gasteria,* and *Astroloba,* electron microscope methods were used. The mature pollen grains from dehisced anthers were incorporated into agar pellets and fixed in 1% osmium tetroxide, buffered at pH 7.2 with 0.1M sodium cacodylate at 4° C for 24 hours. The pellets were then washed several times in distilled water, dehydrated with alcohol and acetone, and embedded in Epon 812. Sections were cut with glass knives on an LKB ultramicrotome or a Porter-Blum ultramicrotome and were examined and micrographed with an RCA or a Philips microscope (Majumdar 1972; Majumdar and Lowry 1971a, b).

In Chapter 10 the development of callus tissues on culture media in *Haworthia* and their use to study the effects of mutagens, herbicides, and carcinogens on chromosomes, cells, and cell organelles will be reported; a brief summary of the methods used to grow tissue cultures might appropriately be included here.

The medium frequently used is a modified White's medium and comprises six major elements, six trace elements, ferric chloride, thiamine hydrochloric acid, pyridoxine hydrochloric acid, niacin, indole-3-acetic acid, kinetin, coconut milk, sucrose, and agar. The mixture is poured into culture tubes which are then covered with aluminum foil, sterilized in an autoclave, and kept in a cool, dark place until used. A more detailed account of this method has been published in several articles (Majumdar 1970a; Majumdar 1970b; Majumdar and Castellano 1977).

Chapter Four

KARYOTYPES

4.1 Camera lucida drawing of chromosomes from the root tip of *Haworthia viscosa* var. *quaggaensis*.

A useful and important device for studying cytogenetics, especially for comparing related species and genera to detect chromosome aberrations and evaluate phylogenetic relationships and evolutionary trends, is the karyotype. It is actually a picture of the chromosomal complement of an individual plant or animal or of a related group of such individuals as seen in somatic metaphase. It is important because it reveals clearly and in readily comprehended fashion the various attributes of the complement: the number of chromosomes; the absolute size of the chromosomes; the relative size of the different chromosomes of the same set; the position of the centromeres on each of the chromosomes; the relative lengths of the arms; the number of metacentric, acrocentric, and telocentric chromosomes; the position of the secondary constriction or constrictions; the number and position of satellites; the position of heterochromatic regions; the number of B chromosomes; and any unusual structural features that may be present.

The use of the karyotype is predicated on the assumption that it is constant within limits; it does not change capriciously in an individual at different times of life, and it is constant for large groups of related organisms. It can change apparently spontaneously and can also be changed by certain environmental factors such as ionizing radiation, but its normal constancy lends significance to these changes. Its constancy is also shown by the fact that centromeres do not arise *de novo,* as was once thought, and that the ends of the chromosomes are permanent structures and have definite properties. Also, since the centromeres are the organs of locomotion of the chromosomes, every chromosome or piece of chromosome must have one and no more than one centromere, unless it has a diffuse centromere, if it is to function normally and properly at all divisions. The karyotype is considered to be a definite species character and has been recognized as such for a long time.

The karyotype is a picture of the entire chromosomal complement of an individual. Formerly it was depicted by drawing the chromosomes with a camera lucida or similar piece of optical equipment (Fig. 4.1); recently this method has been used much less often, and the chromosomes today are more frequently photographed. The photograph of each individual chromosome is then cut out and the chromosomes are matched in pairs and pasted down in one or more

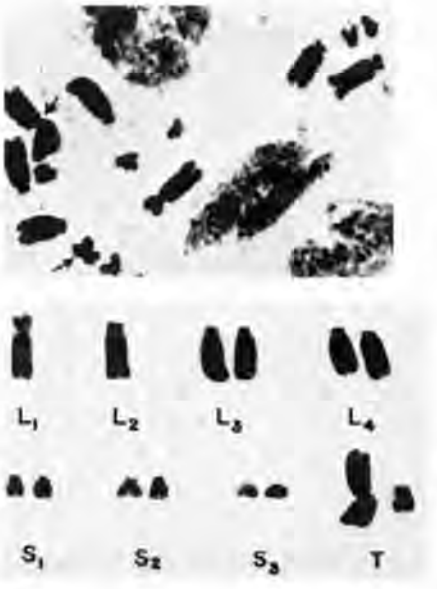
rows (Fig. 4.2). Sometimes each chromosome is carefully measured and a drawing is made of it — not as it would look under the microscope but in a purely stylized way, as a straight line, often fairly thick. Such a drawing of all the chromosomes in the complement is called an idiogram and is often more useful than a karyotype for comparing the chromosomes of one species with those of another (Fig. 4.3). Idiograms or less diagrammatic drawings are often used to compare the chromosomal complements of related genera or species (Figs. 4.4, 4.5).

The karyotypes of the various species of the Aloineae are remarkably uniform. The few exceptions are of the nature of aberrations. The haploid complement typically consists of seven chromosomes, of which four are long and three are much shorter. All the chromosomes are acrocentric or submetacentric, but the length of the short arm is greater in some chromosomes than in others. In all diploid plants there are minor variations, of course, such as secondary constrictions and satellites, but in general the karyotypes are similar. Recently Cutler and Brandham (1977) reported that in several closely related species of *Aloe* the long chromosomes are only a little longer than half as long as those in most of the species of the genus. This condition occurs in *A. tenuior* Haw., *A. ciliaris* Haw., and *A. tidmarshii* (Schoenl.) Müller (= *A. ciliaris* f. *tidmarshii* [Schoenl.] Res.) and was observed strikingly in an artificial hybrid between *Gasteria lutzii* and *Aloe tenuior* var. *rubriflora*. The chromosomes of the two species involved in this cross are similar except for the length of the long arms, and Cutler and Brandham found that this difference in chromosome length can be recognized with ease even in crosses between species within the genus *Aloe*. The short chromosomes of all species are so short that they cannot be measured conveniently; whether there is a difference among the different species comparable to that of the long arms, Cutler and Brandham could not learn.

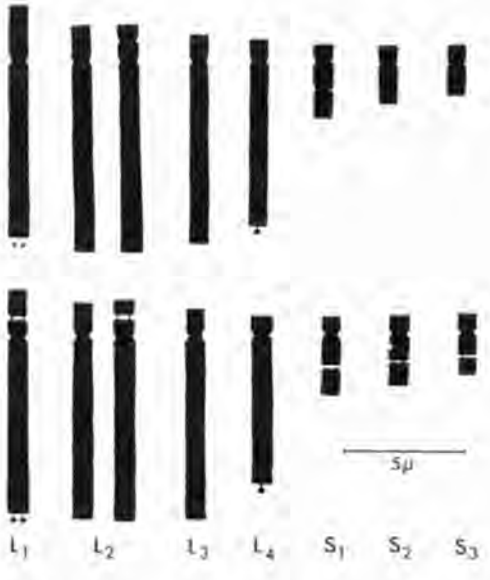
Apparently the first description of the chromosome complement of the Aloineae was published by H. A. C. Müller for *Aloe hanburyana* Naud. (= *A. striata* Haw.). He stated that there were four chromosome pairs measuring 15-16 μm in length, one pair 6 μm long, and two pairs about 4 μm long. Comparing them with the chromosomes of *Najas*, he pointed out that they were not V-shaped but were bent like a hook at one end.

Taylor (1924) described meiotic and mitotic chromosomes and also reported there were four long and three short ones in the haploid complement. He was puzzled by the fact that in at least two of the three pairs of short chromosomes there were two constrictions, but he described accurately the place of spindle-fiber insertion in all seven chromosomes and pointed out that his ideas agreed with the figures of Sakamura (1920). In a later paper Taylor (1925a) clarified this point, stating that "there is one type of constriction related to fiber-attachment and another which is independent."

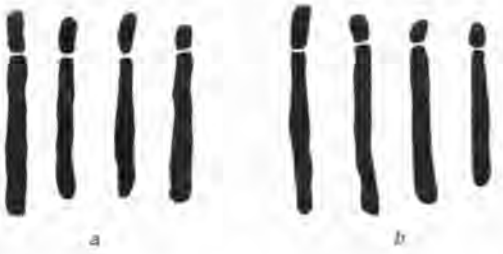
Taylor (1925b) described the karyotypes of *Gasteria*, *Aloe*, and *Haworthia* and compared them. The three short chromosomes seemed to differ little, if any, in the three genera. In *Gasteria* three of the long chromosomes had



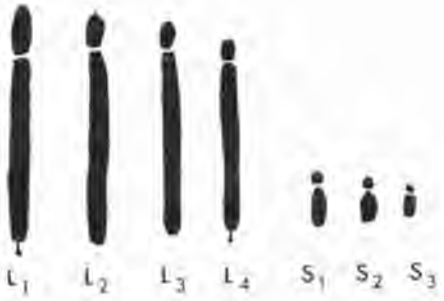
4.2 Photomicrograph of the root-tip chromosomes of *Haworthia attenuata*; arrows and T indicate translocated chromosomes. (*Can.J.Genet.Cytol.*, vol. 9)



4.3 Idiogram showing secondary constrictions in chromosomes of *Haworthia tortuosa* var. *curta*: (*top*) normal; (*bottom*) after cold treatment. (*J.Hered.*, vol. 58)



4.4 Diagram of the long chromosomes of (*a*) *Aloe*, (*b*) *Gasteria*.



4.5 Diagram of the seven haploid chromosomes of *Haworthia*.

4.6 Chromosomes of *Haworthia herrei* var. *herrei* showing satellites at the ends of three chromosomes.

subterminal centromeres while the fourth had "this feature removed from the end by a considerable space." This last chromosome also bore a satellite at the distal end of the long arm. In *Aloe arborescens* Mill. and *A. saponaria* Haw. only one of the long chromosomes had a subterminal centromere; the three others had the centromere "quite a little removed from the end, and resembled in this that pair which bore the satellites in *Gasteria*." However, in *Aloe* the long chromosome with the subterminal constriction is generally one with a satellite. The present writers have confirmed these observations for many species. In *Haworthia cymbiformis* Haw. (var. *obesa* Baker?) the chromosomes resemble those of *Gasteria* except that the chromosome with the centromere a considerable distance from the end does not have a satellite and two of the three other chromosomes have two constrictions. The constriction farther from the end toward which they lie is the centromere; the other constriction shows considerable variation in appearance, which Taylor ascribes to fixation. Actually, in *Haworthia* there is species variation in the satellites and in the secondary constrictions. Figure 4.6 shows the chromosomes of *H. herrei* var. *herrei* with satellites on three chromosomes. Taylor suggests that "it is perhaps not unreasonable to expect that a grouping of the species based on chromosome characters would in general correspond to that adopted by the systematist." Thus early in the cytological study of the Aloineae the possible evolutionary significance of chromosome morphology was recognized.

When Taylor was making his studies, Ferguson was independently studying the chromosomes of the Aloineae at the University of London. She found constrictions but could not discern any patterns of evolutionary importance. As did Taylor, she found that one long chromosome has a constriction about one-third the distance from one end; she did not find, as he did, that in species of *Aloe* this situation was found in three of the four long chromosomes. She could draw no conclusions about the origin of the chromosome pattern of the Aloineae from any of the arrangements previously suggested for other members of the Liliaceae.

Although Taylor, Ferguson (1926), and some other investigators described the diploid complement as comprising eight long and six short chromosomes, Newton (1924) described it as consisting of eight long and four short chromosomes and two of intermediate length. In reality the two "intermediate" chromosomes are much closer in size to the short ones than to the long ones.

Four long and three short chromosomes were found in the haploid complement of *Aloe grandidentata* by Marshak (1934) and in *A. striata* by Sax (cited by Marshak). In "*A. vera*" the complement consisted of three long, one intermediate, and three short ones and, as his figures clearly show, this is a real intermediate (Marshak 1934). (See further discussion of this point and the observations of Vig and Brandham in Chapter 8.) It is not clear whether this plant is *A. barbadensis* Mill. (= *A. vera* "L.") or *A. vera* Mill., since the authorship of the species is not stated; from the description of its geographic distribution it appears to be the former. In twelve species of *Gasteria* and three of *Haworthia*, Marshak (1934) also found

TABLE 4.1 Total Chromosome Length and Short Arm Length of Chromosomal Types of *Aloe* as Described by Müller (1945)

	a		*b*		*c*		*d*		*e* and *f*		*g*	
	Total Length	Short Arm	Total Length	Short Arm	Total Length	Short Arm	Total Length	Short Arm	Total Length	Short Arm	Total Length	Short Arm
Average	15.16	3.67	14.72	2.44	14.01	2.26	13.05	1.82	4.24	0.99	4.16	1.02
Std. dev.	2.01	0.35	1.79	0.38	1.67	0.29	1.62	0.21	0.4	0.05	0.49	0.16
Std. error	0.30	0.05	0.26	0.06	0.25	0.04	0.24	0.03	0.06	0.01	0.07	0.02

four long and three short chromosomes. No satellites, no double constrictions, and no size differences in the short areas of the long chromosomes were observed; but this lack is explained on the basis that Taylor studied somatic mitosis whereas Marshak was working with the microspore division in the pollen grain. In *Astroloba (Apicra) congesta* Marshak found considerable variability in chromosome number, but the usual number appeared to be six long and three short chromosomes in the haploid complement. The significance of this observation is questionable, since this complement does not seem to be corroborated by other observations; Marshak himself stated that it "is doubtful because of the marked variability of the pollen observed."

Several investigators have assigned symbols to the chromosomes of the Aloineae. Sato (1937), for example, used L_1 to designate the long chromosome with the longest short arm; it generally has satellites at the end of the long arm, "except in some species of *Aloe*." Similarly, L_4 represents the long chromosome with the shortest short arm "generally having satellites at the distal arm." The two long chromosomes with short arms shorter than those in L_1 but longer than those in L_4 and which "in *Aloe* rarely have satellites" are L_2 and L_3, but Sato fails to distinguish between them. The three short chromosomes with subterminal constrictions are S_1, S_2, and S_3; the last occasionally has a satellite on the short arm in *Aloe*.

Müller (1945) divided the chromosomes of *Aloe* into seven types, a through g. The a-chromosome is the longest and has the longest short arm. The b-type is almost as long but the short arm is considerably shorter. The c-type is shorter than the other two and has a shorter short arm, although the short arm is only very little shorter than that in the b-chromosome. The d-type is the shortest of the long chromosomes and has the shortest short arm. The three short chromosomes are very much alike. Two are slightly longer than the third but have a short arm that is almost the same size and just barely shorter; these are the e and f chromosomes, and they are generally grouped together. The third is the g-chromosome. The average lengths in micrometers of these chromosomes are shown in Table 4.1. (The word micrometer is used here with its new meaning to replace the unit of measurement for a number of decades called a micron.)

Not all cytologists feel they can distinguish the four long chromosomes as easily as Müller felt he could in *Aloe*. Snoad (1951a) studied *Aloe, Gasteria,* and *Haworthia* and identified four chromosomal types. Type A represented the longest of the four long chromosomes and the one that had

the longest short arm, and type C was the shortest long chromosome and had the shortest short arm. These types are easily identified and are the a and d types of Müller. The two other long chromosomes could not be separated by Snoad, who grouped them together as type B; they are about the same length, and the small arms seem to be identical as shown in the drawings in Snoad's paper. Type D represented all the small chromosomes which cannot in Snoad's drawings be distinguished one from another; none shows any secondary constrictions as seen by Taylor (1925b) and others. It is interesting to note that Snoad found no generic differences in the lengths of the short arm of the long chromosomes; Taylor (1925b), on the basis of too few observations, found that only one chromosome had a long short arm in *Gasteria,* whereas three did in *Aloe*.

Riley and Majumdar (1966b) grouped the chromosomes of *Haworthia* into seven basic types, maintaining that the type B chromosomes of Snoad (1951a) can be distinguished from one another. The basic types (see Fig. 4.5) are:

L_1 — long; longest short arm
L_2 — long; short arm shorter than in L_1
L_3 — long; short arm somewhat shorter than in L_2
L_4 — long; shortest short arm
S_1 — short; submedian to subterminal centromere
S_2 — short; slightly shorter than S_1; submedian to subterminal centromere
S_3 — short; shortest chromosome; submedian to subterminal centromere

Chromosomes such as the long translocation chromosome with median centromeres, and intermediate-sized chromosomes (Riley, Majumdar, and Hammack 1967), short metacentric chromosomes (Riley and Majumdar 1968), the shortened long chromosome of *H. fulva* (Riley and Majumdar 1968), spontaneous aberrations (Vig 1968), and isochromosomes (Brandham 1970) — all of which are undoubtedly aberrant types resulting from translocations — are not included as part of a normal basic karyotype, although they may be new permanent karyotypes that are evolving or have just evolved. Studies by Sharma and Mallick (1965) and Majumdar and Riley (1967) showed that minute structural differences seem to be present in many species, but these cannot constitute different karyotypes.

In all the genera of the Aloineae that have been studied, certain structural features of the chromosomes are often found, such as secondary constrictions and satellites (Fig. 4.6). *Gasteria* chromosomes (Riley, Hammack, and Majumdar 1968) show the same basic seven types although the short chromosomes are usually more difficult to differentiate from one another. Again measurements of chromosomes seem to show small differences between species.

Secondary constrictions other than the "threads" that connect satellites are occasionally seen on various chromosomes, but they are usually not prominent. Cold treatment, however, revealed some that are not normally seen and made the normal ones more conspicuous. In *Haworthia tortuosa* var. *curta,* chromosomes L_1, L_3, L_4, S_2, and S_3 do not often show constrictions in untreated plants; but a constriction is usually seen in one L_2 chromosome (in the

short arm) and in the long arm of both members of S_1 (Riley and Majumdar 1967). When cold-treated, the constriction in the one L_2 and both S_1 chromosomes became very clear and new constrictions not previously seen were revealed in the L_1, S_2, and S_3 pairs (see Fig. 4.3). In the first they were in the short arms and in the last two, in the long arms. The constriction found in the short arm of one L_2 chromosome was also found in *Gasteria schweicherdtia* and *G. conspurcata*. Oddly, it was heterozygous in each plant and was not found in five other cold-treated species. A secondary constriction in this chromosome arm is beautifully illustrated in a drawing by Darlington and Kefallinou (1957) in both members of the L_2 chromosome of *Gasteria undulata,* a species not listed in Jacobsen (1960). Although the lengths of the long and short chromosomes are generally roughly equal among the various genera, there is one distinct exception, as shown by Cutler and Brandham (1977). This situation is especially noticeable in an intergeneric hybrid and is discussed in Chapter 11.

Recently some studies on *Astroloba* and *Poellnitzia* by Majumdar (1968), Brandham (1971), and Majumdar and Riley (1972) showed that all the diploid species except *As. herrei* have the usual karyotype of $2L_1 + 2L_2 + 2L_3 + 2L_4 + 2S_1 + 2S_2 + 2S_3$ chromosomes and that triploid and tetraploid plants have the same chromosomes but in multiples. In *As. herrei* the formula is $2L_1 + 4L_2 + 2L_4 + 2S_1 + 2S_2 + 2S_3$.

One of the characteristic features of some chromosomes is a segment that occurs usually at the distal end of an arm (generally the short arm) and is separated from it by a secondary constriction, which is part of the chromosome but is often thinner than the remainder and appears as an unstained or lightly stained thread. This constriction is a nucleolus organizer and is the place where a nucleolus is formed at telophase; the ball-like segment is simply the part of the chromosome distal to the constriction. This chromosomal segment was termed a "Trabant" or "satellite" by Navashin (1912). Together the satellite and the secondary constriction form the "satellite region," but apparently only the constriction is of much functional importance. The satellite may be less than one-half the diameter of a chromosome or may be greater than one-half. It may be globular or linear; although it is usually terminal in position, it may occasionally be intercalary. The fact that the nucleoli arise on the satellite chromosomes and below the satellites was pointed out by Heitz (1931); he termed this chromosome the "SAT-chromosome," based on the initials of the expression "sine acido thymonucleinico" or "without thymonucleic acid" (the modern name of which is deoxyribonucleic acid or DNA). The term refers to the secondary constriction, which often appears unstained with the Feulgen stain.

Resende and Rijo (1948) mention that this region may show great variability, ranging from totally achromatic to chromatic, or from having no DNA to having it. It also varies in width, ranging from filiform to wider than the rest of the chromosome. Resende, Lemos-Pereira, and Cabral (1944) named these contracted regions the "nucleolar olistherozones" and the chromatic substances of which they consist the "nucleolar olistherochromatin."

Some of the earlier karyotype studies were concerned primarily with the number and position of these satellites, and there was considerable disagreement in the observations. Taylor's work has been mentioned, and Ferguson (1926) found satellites in only one variety of *Aloe arborescens*. Several investigators have disagreed on the number of satellites and nucleoli that are present. Heitz (1931, 1935) found there were four satellites and four nucleoli in *Aloe arborescens* and *A. ferox,* observations that fit in with the theory then being propounded that satellites are connected with nucleoli and nucleolus organizers. However, Fernandes (1931, cited in Resende 1936) found two satellites in only three *Aloe* hybrids of the ten he studied, and Tuan (1931) in the haploid complement of a *Gasteria* plant found one. Geitler (1935) found one satellite in the haploid complement of one species of *Aloe,* one of *Astroloba,* and four of *Gasteria;* but, in contradiction to the theory of nucleolar organizers, there seemed to be two nucleoli. Heitz's results were corroborated by Resende (1936), who found 99 species of the Aloineae with four satellites and four nucleoli and three species with two of each. An extension of this study was made (Resende 1937a) with 151 species of Aloineae and 34 species of other genera and families. Most species had four satellites, but some had three, two, or one.

Sato (1937) also described the satellite situation. He found that (1) the satellite patterns are not so definitely distinct according to genera as Taylor (1925b) emphasized and (2) not all plants always have four satellites and four nucleoli as Resende (1936) reported. Sato studied root-tip mitosis and considered that anaphase was best for observing the satellites. In *Aloe* the species differed according to the number, size, and position of the satellites but the number of satellited chromosomes agreed with the number of nucleoli. There were four satellited chromosomes and four nucleoli in six species. In one of the species the chromosomes were $2L_1$ and $2L_4$; in one they were $2L_2$ and $2L_4$; in one they were $2L_3$ and $2L_4$; in two they were $2L_4$ and $2S_3$; and in one they were $1L_1 + 2L_4 + 1S_3$. There were three satellited chromosomes and three nucleoli in four plants. One plant had $2L_1$ and $1L_4$, and one had $2L_4$ and $1S_3$. Two plants had two satellited chromosomes and two nuclei, and the chromosomes were both L_4. One plant had four chromosomes with satellites ($1L_1 + 1L_3 + 2L_4$) but had five nucleoli, one of which was very small. The pattern of satellites was more regular in *Gasteria* and *Haworthia*. In the former, twelve species or hybrids had four satellited chromosomes ($2L_1$ and $2L_4$) and four nucleoli; three had three SAT-chromosomes (two with $1L_1$ and $2L_4$ and one with $2L_1$ and $1L_4$) and three nucleoli. One plant, an intergeneric hybrid with *Aloe,* had six SAT-chromosomes ($2L_1$ and $4L_4$) and six nucleoli, and one tetraploid had eight such chromosomes ($4L_1$ and $4L_4$) and eight nucleoli. One plant had six SAT-chromosomes and nucleoli, but two of the chromosomes had a satellite at each end, apparently as the result of a translocation. In general the karyotypes were similar, but there were size differences among the chromosomes of the different species. Ten *Haworthia* plants had four SAT-chromosomes and four nucleoli, and all the chromosomes were L_1 and L_4; three plants

had three such chromosomes ($1L_1$ and $2L_4$ or $2L_1$ and $1L_4$) and three nucleoli. Some of the karyotypes in Sato's work were atypical because of translocations or polyploidy, but they in no way alter our concepts of the basic karyotype of the tribe.

Satellites are a feature of the karyotype, but a study of them often gives unsatisfactory and confusing results. As was pointed out previously, Sato found that ten *Haworthia* plants had four SAT-chromosomes in the diploid complement (chromosomes L_1 and L_4) and three plants had satellites on three of those chromosomes. Such consistency was not observed by Mukerjee and Riley (1961). They found that satellites were present on the short arm of one pair of long chromosomes (not identified) in *H. planifolia* var. *planifolia* and on the long arm of one pair of long chromosomes in an unidentified species of the Rigidae section. In *H. attenuata* var. *caespitosa* two pairs of long chromosomes had satellites on the long arm, but one of these four chromosomes also had a satellite at the end of the short arm. In nine other species or varieties no satellites were observed on any chromosomes. The absence of satellites is of little significance; it may signify they are not present but may also mean they were not clearly fixed. They may be small, and the thread that connects them to the body of the chromosome may be short and may vary in size so much that the satellite may be in close contact with the rest of the chromosome and hence not visible.

Chapter Five

CHROMOSOME NUMBERS

For nearly sixty years many observers have looked at and counted the chromosomes of the Aloineae. Ever since H. A. C. Müller (1912) pointed out that the sporophytic number was 14 in *Aloe striata* Haw. (published as *A. Hanburyana* Naud.), many species of *Aloe, Astroloba* (*Apricra*), *Chamaealoe, Gasteria, Haworthia, Lomatophyllum,* and *Poellnitzia* have been studied. Most of the species have 14 sporophytic chromosomes, but there are some polyploid and some aneuploid plants, especially in *Haworthia*. From time to time lists of chromosome numbers have been compiled, such as those by Suto (1936), Kondo and Megata (1943), Snoad (1951a), and Riley (1959c, 1960, 1961), as well as general works not confined to the Aloineae, such as books and papers by Gaiser (1926, 1930), Darlington and Wylie (1955), and Cave (1956-65), and lists published annually in *Taxon* (Moore 1970-76). Since there has been no summary of the work published since 1961, since a number of papers that include chromosome numbers have appeared during the last decade, and since some chromosome studies have been made but not published, a complete list up to the present time should be published even with the realization that it will probably be out of date upon publication. Tables 5.1-5.5 include these chromosome numbers. The validity of these tables is no greater than the accuracy of the identification of the species, and the names assigned to many of the species used in published records can well be questioned. Unless these names are correct, the chromosome numbers mean little.

Author Abbreviations

In the interest of brevity, sources for the chromosome numbers of the species listed in the tables of this chapter are condensed. Names of authors are abbreviated as follows, and a shortened form of the date of publication is used.

A - Afify
Be - Belling
BbG - Brewbaker & Gorrez
Bn - Brandham
BnJ - Brandham & Johnson
Br - Brinckmann
CBn - Cutler & Brandham
ChS - Chinnappa & Semple
Cl - Caillon
Cm - Camara
DK - Darlington & Keffallinou
Fg - Ferguson
Fn - Fernandes

FnN - Fernandes & Neves
Ga - Gaiser
Ge - Geitler
Gl - Giles
Go - Gioelli
H - Heitz
Ja - Jähnl
Jn - Johnson
Jo - Johansen
Js - Joshi
Ka - Kaul
KnM - Kondo & Megata
Ko - Koshy
Ma - Mathew
MBn - Mathew & Brandham
Me - Mendes
Mj - Majumdar
MjRi - Majumdar & Riley
MkRi - Mukerjee & Riley
Mr - Marshak
MtS - Matsuura & Suto
Muf - F. S. Müller
Muh - H. A. C. Müller
N - Newton
Pl - Pinto-Lopes
PlRe - Pinto-Lopes & Resende
Pr - Propach
Re - Resende
ReA - Resende & Amaral
ReF - Resende & da Franca
ReL - Resende, de Lemos-Pereira, & Cabral
ReM - Resende & Manarte
RePl - Resende & Pinto-Lopes
ReV - Resende & Viveiros
Ri - Riley
RiHMj - Riley, Hammack, & Majumdar
RiI - Riley & Irvin
RiMj - Riley & Majumdar
RiMjH - Riley, Majumdar, & Hammack
RiMk - Riley & Mukerjee
Ro - Rowley
Ru - Rüdenburg
ShC - Sharma & Chatterjee
ShD - Sharma & Datta
ShM - Sharma & Mallick
SiSt - Sinoto & Sato
Sm - Sakamura
Sn - Snoad
Sr - Sutaria
St - Sato
Su - Suto
Ta - Taylor
Tu - Tuan
Vg - Vig
Vv - Viveiros

TABLE 5.1 Chromosome Numbers in *Aloe*

Species	2*n*	Source
Species Listed in Jacobsen (1954) and Reynolds (1950, 1966) or Recently Named		
A. aculeata Pole Evans	14	Ri 59a
A. acutissma H. Perr.	14	Bn 71
A. acutissma var. *antanimorensis* Reyn.	14	Bn 71
A. africana Mill.	14	(See under *A. bainesii* and *A. plicatilis* below.)
A. albida (Stapf.) Reyn. as *Leptaloe albida* Stapf.	14	Muf 45
A. ammophila Reyn.	14	Bn 71
A. amudatensis Reyn.	14	Bn 71
A. andongensis Bak.	14	Bn 71
A. andringitrensis H. Perr.	14	Re 37a
A. angelica Pole Evans	14	Ri 59a
A. antandroi H. Perr.	14	Bn 71; CBn 77
A. arborescens Mill.	14	Ta 25b; Fg 26; Go 30; Re 37a; Muf 41, 45; KnM 43; Sn 51a; Bn 71
as *A. arborescens* var. *frutescens* Link	14	Re 37a
as *A. arborescens* var. *milleri* Bgr.	14	ShM 65
as *A. arborescens* var. *natalensis* (Wood & Ev.) Bgr.	14	Fg 26; Re 37a; ShM 65

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. aristata Haw.	14	Re 37a; St 37, 42; KnM 43; Ri 59a; ShM 65; Bn 71; CBn 77
A. aristata var. *leiophylla* Bak.	14	Re 37a
A. aristata Roem. & Schult.: see *Haworthia aristata* [Table 5.3]		
A. ausana Dtr.	14	Re 37a
A. bainesii Th. Dyer	14	Re 37a (Really *A. africana* acc. to Viveiros, 1949)
A. bakeri Scott Elliot	14	Bn 71
A. ballii Reyn.	14	Bn 71
A. ballyi Reyn.	14	Bn 71
A. barbadensis Mill.	14	Ka 64
as *A. vera* L.	14	Sr 32; Mr 34; Re 37a; ShC 58; Vg 65, 68
as *A. vera* var. *chinensis* Bak.	14	Re 37a; KnM 43
A. barbertoniae Pole Evans	14	Re 37a
A. bellatula Reyn.	14	Bn 71
A. boylei Bak.	14	Muf 45
A. branddraaiensis Groenew.	14	Bn 71
A. brevifolia Mill.	14	Re 37a; St 37, 42; KnM 43; Sn 51a; ShM 65; Bn 71
A. brevifolia var. *depressa* (Haw.) Bak.	14	Re 37a
A. brevifolia var. *postgenita* (R. & S.) Bak.	14	Re 37a
A. brunnthaleri Bgr.: see *A. microstigma*		
A. buettneri Bgr.	14	Bn 71
as *A. paedogona* Bgr.	14	FnN 62
A. bukobana Reyn.	14	Bn 71
A. bulbillifera H. Perr.	14	Re 37a; Bn 71
A. burgersfortensis Reyn.	14	Bn 71
A. calidophylla Reyn.	14	Bn 71
A. cameronii Hemsl.	14	Fg 26; Muf 45; Bn 71
A. camperi Schweinf.	14	Bn 71
as *A. eru* Bgr.	14	Re 37a; DK 57
A. capitata var. *capitata* Bak.	14	St 37, 42
A. capitata var. *cipolinicola* H. Perr.	14	Bn 71
A. castanea Schoenl.	14	Muf 45
A. catengiana Reyn.	14	Bn 71
A. chabaudii Schoenl.	14	Muf 45; Ri 59a
A. chabaudii var. *mlanjeana* Christian	14	Bn 71
A. chortolirioides Bgr.	14	Muf 45
A. christianii Reyn.	14	Bn 71
A. ciliaris Haw.	42	Re 37a, 38; Muf 41, 45; ReA 56; Ri 58, 59a; Br 60; ShM 65; Bn 71
[from 4 different sources]	42	Sn 51a
	>45	Fg 26
	>50	Go 30
A. ciliaris f. mut. *tidmarshii* (Schoenl.) Res.: see *A. tidmarshii*		
A. claviflora Burch.	14	Ri 59a
as *A. schlechteri*	14	Re 37a
A. commixta Bgr.	14	Sn 51a
A. comosa Marl. & Bgr.	14	Ri 59a
A. comptonii Reyn.	14	Ri 59a

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. concinna Bak.: see *A. zanzibarica*		
A. confusa Engl.	14	Muf 45; Bn 71
A. cooperi Bak.	14	Re 37a; KnM 43; Muf 45; Ri 59a; Bn 71
A. cryptopoda Bak.	14	Muf 45; Ri 59a; ShM 65
as *A. pienaarii* Pole Evans	14	Re 37a; Muf 41, 45
A. davyana Schoenl.	14	KnM 43; ShM 65
[6 wild plants; seeds from 34 wild plants]	14	Ri 58, 59a
A. davyana var. *subulifera* Groenew.	14	Muf 45
A. dawei Bgr.	28	Bn 71; CBn 77
A. deltoideodonta var. *candicans* H. Perr.	14	Bn 71
A. descoignsii Reyn.	14	Bn 71
A. dewetii Reyn.	14	Muf 45
A. dichotoma L. f.	14	Re 37a; Ri 59a
A. distans Haw.	14	Bn 71
A. divaricata Bgr.	14	Bn 71
A. dorotheae Bgr.	14	Bn 71; CBn 77
A. dumetorum Mathew & Brandham	14	MBn 77
A. dyeri Schoenl.	14	Muf 45; ShM 65; Bn 71
A. ecklonis S.D.	14	ShM 65
A. elgonica Bullock	28	Bn 71; BnJ 77
	29	BnJ 77
A. erensii Christian	14	Bn 71
A. eru Bgr.: see *A. camperi* Schweinf.		
A. excelsa Bgr.	14	Bn 71
A. ferox Mill.	14	Fn 30, 31; H 35; Re 37a; St 37, 42a; KnM 43; Ri 59a; ShM 65; Bn 71
as *A. supralaevis* Haw.	14	Re 37a
as *A. supralaevis* var. *foliis variegatis*	14	Re 37a
A. forbesii Balf. f.	14	Bn 71
A. fosteri Pillans	14	Muf 45; Ri 59a; Bn 71
A. framesii L. Bol.	14	Ri 59a
A. gerstneri Reyn.	14	Muf 45
A. gigas Res.	35	ReA 56
A. glauca Mill.	14	Re 37a
A. globuligemma Pole Evans	14	Muf 41, 45; Ri 59a
A. gloveri Reyn. & Bally: see *A. hildebrandtii* Bak.		
A. gracilis Haw.	14	ReA 56; Br 60
as *A. laxiflora* N. E. Br.	14	Re 37a; Muf 41, 45
A. gracilis var. *minima*: see *Gasteria gracilis* var. *minima* [Table 5.2]		
A. graminicola Reyn.	14	Bn 71
A. grandidentata S.D.	14	Mr 34; Re 37a
A. greatheadii Schoenl.	14	ShM 65
A. greenwayi Reyn.	14	Bn 71
A. guerrai Reyn.	14	Bn 71
A. hanburyana Naud.: see *A. striata*		
A. harlana Reyn.	14	Bn 71
A. hereroensis Engl.	14	Re 37a; Muf 41, 45; Ri 59a
A. hildebrandtii Bak. as *A. gloveri* Reyn. & Bally	14	Bn 71
A. humilis (L.) Mill.	14	St 37, 42; Bn 71
	21	ShM 65

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. humilis var. *echinata* (Willd.) Bak.	14	Ge 35
as *A. humilis* var. *equinata* Bak.	14	Re 37a
A. ibityensis H. Perr.	14	Bn 71
A. immaculata Pillans	14	Bn 71
A. integra Reyn.	14	Muf 45
A. isaloensis H. Perr.	14	Bn 71
A. jacksonii Reyn.	28	Bn 71
A. jucunda Reyn.	14, 21	Bn 71
A. juttae Dntr.: see *A. microstigma*		
A. karasbergensis Pillans	14	Ri 59a
A. keayi Reyn.	14	N 70; Bn 71
A. khamiesensis Pillans	14	Bn 71
A. kniphofioides Bak. as *A. marshalii* Wood & Evans		
A. krapohliana Marl.	14	Re 37a
A. lateritia Engl.	14	Re 37a; Bn 71
A. latifolia Haw.: see *A. saponaria*		
A. laxiflora N.E. Br.: see *A. gracilis*		
A. laxissima Reyn.: see *A. transvaalensis*		
A. lettyae Reyn.	14	Muf 41, 45; Bn 71
A. lineata (Ait.) Haw.	14	Muf 41, 45
A. lineata var. *muiri* (Marl.) Reyn. as *A. muiri* Marl.	14	Re 37a
A. littoralis Bak.	14	Bn 71
as *A. rubrolutea* Schinz.	14	Re 37a; Muf 41, 45; Ri 59a; ShM 65
A. longibracteata Pole Evans	14	Re 37a; Ri 59a
A. longistyla Bak.	14	Re 37a; ShM 65; Bn 71
A. lutescens Groenew.	14	Bn 71
A. macracantha Bak.: see *A. saponaria*		
A. macrantha: see *A. saponaria*		
A. macrocarpa Tod.	14	St 37, 42
A. macrocarpa var. *major* Bgr.	14	N 70
A. macrosiphon Bak.	14	Bn 71
A. madecassa H. Perr.	14	Re 37a
A. marlothii Bgr.	14	Re 37a; Ri 59a; ShM 65
A. marshalii Wood & Evans: see *A. kniphofioides*		
A. mawii Christian	14	Bn 71
A. mcloughlinii Christian	14	Bn 71
A. megalacantha Bak.	14	Bn 71
A. melanacantha Bgr.	14	Re 37a; Muf 45
A. microcantha Haw.	14	Muf 41, 45
A. microstigma S.D.	14	Re 37a; Ri 59a; ShM 65
as *A. brunnthaleri* Bgr.	14	Pr 34; Re 37a; ShM 65
as *A. juttae* Dntr.	14	Re 37a
A. millotii Reyn.	14	Bn 71
A. mitriformis Mill.	14	Ri 59a
as *A. mitriformis* Haw.	14	Re 37a
as *A. mitriformis* var. *typica*	14	Re 37a
as *A. parvispina* Schoenl.	14	Re 37a
A. mitriformis var. *commelinii* (Willd.) Bak.*	14	Re 37a
A. mitriformis var. *flavispina* (Haw.) Bak.*	14	Re 37a
A. mitriformis var. *spinulosa* (S.D.) Bak.*	14	Re 37a

*Doubtful varieties according to Reynolds (1950).

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. mudenensis Reyn.	14	Ri 59a
A. muiri Marl.: see *A. lineata* var. *muiri*		
A. mutabilis Pillans	14	Muf 41, 45
A. myriacantha (Haw.) Roem. & Schult.	14	Bn 71
A. mzimbana Christian	14	Bn 71
A. ngobitensis Reyn.	14	Bn 71
A. nubigena Groenew.	14	Muf 45
A. paedogona Bgr.: see *A. buettneri*		
A. palmiformis Bak.	14	Bn 71
A. parvibracteata Schoenl.	14	Ri 59a; Bn 71
as *A. pongolensis* Reyn.	14	Re 37a
A. parvispina Schoenl.: see *A. mitriformis*		
A. pattersonii Mathew	14	Ma 78
A. pearsonii Schoenl.	14	Re 37a
A. peckii Bally & Verdoorn	14	Bn 71
A. percrassa Tod.	14	Re 37a
A. petricola Pole Evans	14	Ri 59a
A. pienaarii Pole Evans: see *A. cryptopoda*		
A. pirottae Bgr.	14	Bn 71
A. plicatilis (L.) Mill.	14	Fn 30, 31; Re 37a; Sn 51a; Ri 59a; Bn 71. (Resende's plant is probably *A. africana* acc. to Viveiros, 1949)
A. pluridens Haw.	14	Fg 26; Re 37a; Muf 45; Bn 71
A. polyphylla Schoenl.	14	Ri 59a
A. pongolensis Reyn.: see *A. parvibracteata*		
A. pratensis Bak.	14	Ri 59a; Bn 71
A. pretoriensis Pole Evans	14	Re 37a; Muf 41, 45
A. pruinosa Reyn.	14	Muf 41, 45
A. pubescens Reyn.	14	Bn 69b, 71; BnJ 77
A. purpurascens Haw.: see *A. succotrina*		
A. rabaiensis Rendle	14	Bn 71; BnJ 77
A. ramosissima Pillans	14	Ri 59a; Bn 71
A. rauhii Reyn.	14	Bn 69a, 71
A. reitzii Reyn.	14	Muf 41, 45; Ri 59a
A. rigens Reyn. & Bally	14	Bn 71
A. rubrolutea Schinz.: see *A. littoralis*		
A. rupestris Bak.	14	Ri 59a
A. rupicola Reyn.	14	Bn 71
A. saponaria (Ait.) Haw.	14	Ta 25b, 25c; Re 37a; St 37, 42; Sn 51a; ShM 65
as *A. latifolia* Haw.	14	St 37, 42
as *A. macracantha* Bak.	14	Jo 39
as *A. macrantha*	14	Su 36 (attrib. to Johansen)
A. saponaria var. (?)	14	Re 37a
A. saundersiae (Reyn.) Reyn. as *Leptaloe saundersiae*	14	Muf 45
A. schelpei Reyn.	14	Bn 71
A. schlechteri Schoenl.: see *A. claviflora*		
A. schweinfurthii Bak.	14	N 70

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. schweinfurthii var. *labworana* Reyn.	14	N 70
A. scobinifolia Reyn. & Bally	14	Bn 71
A. simii Pole Evans	14	Muf 45
A. sinkatana Reyn.	14	Bn 71
A. somaliensis W. Watson	14	Bn 71
A. speciosa Bak.	14	Re 37a; St 37, 42; Ri 59a
A. squarrosa Bak.	14	Bn 71
A. steudneri Schweinf.	14	Re 37a
A. striata Haw.	14	Sax in Mr 34; Re 37a; KnM 43; Muf 45; Ri 59a; ShM 65
as *A. hanburyana* Naud.	14	Muh 12
A. striatula Haw.	14	Fn 30, 31; Re 37a; Muf 41, 45; Sn 51a; Ri 59a; Bn 71
A. striatula var. *caesia* f. *caesia* Reyn.	14	ReA 56; Br 60
A. striatula var. *caesia* f. *conimbricensis* Res.	14	ReA 56; Br 60
A. succotrina Lam.	14	Re 37a; Ri 59a; Bn 69a, 71
as *A. purpurascens* Haw.	14	Be 28; Ga 30; Re 37a
A. suffulta Reyn.	14	Bn 71
A. suprafoliolata Pole Evans	14	Muf 41, 45
A. supralaevis Haw.: see *A. ferox*		
A. supralaevis var. *foliis variegatis*: see *A. ferox*		
A. suzannae R. Decary	14	Bn 71
A. tenuior Haw.	14	Re 37a; Muf 45; Sn 51a; ReA 56; Ri 59a; Bn 71
A. tenuior var. *decidua* Reyn.	14	Muf 45
A. tenuior var. *rubriflora* Reyn.	14	Muf 41, 45; ReA 56; Bn 71; CBn 77
A. thraskii Bak.	14	Muf 41, 45; Bn 71
A. tidmarshii (Schoenl.) Müller	14	Muf 45
as *A. ciliaris* f. mut. *tidmarshii* (Schoenl.) Res.	42	Sn 51a
A. tororoana Reyn.	14	Bn 71
A. transvaalensis O. Kuntze as *A. laxissima* Reyn.	14	Muf 41, 45
A. trichosantha Bgr.	14	Bn 71
A. turkanensis Christian	14	Bn 71
A. ukambensis Reyn.	14	Bn 71
A. vaombe Decorse & Poisson	14	Bn 69a, 71
A. variegata L.	14	Re 37a; St 37, 42; KnM 43; Sn 51a; ShM 65; Bn 71; CBn 77
A. vera L.: see *A. barbadensis*		
A. vera var. *chinensis* Bak.: see *A. barbadensis*		
A. verdoorniae Reyn.	14	Ri 59a; ShM 65; Bn 71
A. verecunda Pole Evans	14	Muf 45
A. veseyi Reyn.	14	Bn 71
A. vogtsii Reyn.	14	Muf 45; Bn 71
A. vossii Reyn.	14	Muf 41, 45; Ri 59a; Bn 71
A. wickensii Pole Evans	14	Muf 41, 45; Ri 59a; ShM 65
A. wickensii var. *lutea* Reyn.	14	Muf 41, 45

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. zanzibarica Milne-Redhead as *A. concinna* Bak.	14	Re 37a
A. zebrina Bak.	14	Fn 30, 31; Re 37a; KnM 43; Bn 71
Horticultural Varieties		
A. arborescens var. *frutescens* (S.D.) Link.	14	Re 37a
A. salaris Hort.	14	Sn 51a
A. spuria Bgr. (cult. in La Mortola. Hybrid?)	14	St 42; ShM 65
A. straussii Bgr. (garden plant)	14	ShM 65
Hybrids or Putative Hybrids		
A. arborescens Mill. X *A. chortoliri-oides* Bgr.	14	Muf 45
A. bortiana Terrac. f.	14	St 37, 42
A. caesia Salm. (= *A. arborescens* X *A. ferox*)	14	Go 30; Re 37a
A. commutata Tod. (= *A. grandidentata* X *A. saponaria?*)	14	ShM 65
A. ferox var. *galpinii* Reyn. X *A. microstigma* Salm.	14	Muf 45
A. globuligemma Pole Evans X *A. castanea* Bak.	14	Muf 45
A. humilis X *A. spinosissima* Hort.	14	Fn 30, 31; Re 37a
A. X*lisbonensis* (= *A. ciliaris*, 6*x* X *A. gracilis*, 2*x*)	28	ReA 56; Br 60
A. luteobrunea X Bgr. (= *A. ferox* Mill. X *A. principis* (Haw.) Stearn as *A. thraskii* de Willd.	14	Re 37a
A. X*paxii* Terrac. f.	14	Fn 30; St 37, 42
A. pseudopicta X Bgr. [natural hybrid near *A. obscura* Mill.]	14	ShM 65
A. runcinata Bgr. (= *A. obscura* Bgr. [non Mill.] ex Schoenl.)	14	Re 37a; ShM 65
as *A. obscura* Bgr.	14	Re 37a
A. salmdyckiana Roem. & Schult. (= *A. arborescens* X *A. ferox*)	14	Re 37a
A. saponaria X *A. macracantha?*	14	Me 50
A. X*schimperi* Tod. (*A. saponaria* X *A. striata*)	14	Re 37a; St 37, 42; A 45; Sn 51a
A. speciosa X *A. striata*	14	Re 37a
A. speciosa X *A. supralaevis*	14	Re 37a
A. spinosissima X Hort. (= *A. humilis* var. *echinata* X *A. arborescens* var. *pachythyrsa*)	14	Re 37a; Sn 51a
A. striata X *A. saponaria*	14	Re 41
A. striata X *A.* sp. (?)	14	KnM 43
A. todari X var. *praecox* Borzi (*A. humilis* X *A.* sp.)	14	Go 30
Unidentified Plants		
A. sp. [5 plants]	14	Re 37a
A. sp. [1 plant]	14	Me 50
A. sp. [16 plants]	14	Sn 51a

TABLE 5.1 Chromosome Numbers in *Aloe* (*continued*):

Species	2*n*	Source
A. sp. [5 plants]	14	Bn 71
A. sp. [1 plant]	14	Ri 59a
A. sp. [1 plant]	28	Bn 71
A. sp. [1 plant]	14	MtS 35
Aloe [73 unidentified taxa]	14	Bn 69a
Species and Varieties Not Listed in Jacobsen (1954) or Reynolds (1950, 1966)		
A. abyssinica [n. a.]	14	Fg 26
A. bergeriana Dintr.	14	Re 37a
A. coccinea?	14	Sn 51a
A. cristata	14	Fg 26
A. globosa	14	ShM 65
A. gracilis var. *minima*	14	St 37
A. grandis	14	Fg 26
A. humilis Haw., non (L.) Mill.	14	Re 37a
A. longifolia Haw.	14	Sn 51a
A. malesticum	14	ShM 65
A. pringelii Jacobsen	14	ShM 65
A. stricta	14	St 37, 42
A. tenuifolia Lam.	42	Sn 51a
A. varvari	14	Go 30
A. winteri Bgr.	14	Fn 30, 31; ShM 65
A. sp. "juvenna"	28	Bn 71

TABLE 5.2 Chromosome Numbers in *Gasteria*

Species	2*n*	Source
Species Listed in Jacobsen (1954, 1970)		
G. acinacifolia (Jacq.) Haw.	14	St 37, 42; Ri 45, 59b, 59d; Bn 71
G. angustiarum v. Poelln.	14	Ri 59b
G. angustifolia (Ait.) Haw.	14	Ge 35; Ri 59d; Bn 71
G. angustifolia var. *laevis* (S.D.) Haw.		
as *G. laevis* Haw.	14	Sn 51a
G. armstrongii Schoenl.	14	Re 37a; St 37; Ri 45, 59d; Sn 51a
	15	St 42
[3 plants]	14	Bn 71
G. batesiana G. Rowley	14	Ro 51; Bn 71
G. beckeri Schoenl.	14	Sn 51a; Bn 71
[8 plants]	14	Ri 58, 59b
G. bicolor Haw.	14	Bn 69a, 71
G. brachyphylla: see *G. brevifolia*		
G. brevifolia Haw.	14	Mr 34; Re 37a; St 37, 42; Ri 45; 59b, 59d
as *G. brachyphylla*	14	St 37, 42
G. caespitosa v. Poelln.	14	Bn 71
[3 plants]	14	Ri 59b
G. candicans Haw.	14	Bn 69a, 69b, 71
G. carinata (Mill.) Haw.	14	Mr 34; Re 37a; St 37, 42; Ri 59d; Bn 71; CBn 77
as *G. carinata* Duval	14	Sn 51a
G. colubrina N. E. Br.	14	Sn 51a

TABLE 5.2 Chromosome Numbers in *Gasteria* (*continued*):

Species	2*n*	Source
G. conspurcata (S.D.) Haw.	14	Ri 59d; RiMj 67; RiHMj 68
as *G. disticha* var. *comprucata*	14	St 37, 42
as *G. lingua* var. *conspurcata*	14	Fg 26
G. croucheri (Hook. f.) Bak.	14	Sn 51a; ShM 65; Bn 71
G. decipiens Haw. [2 plants]	14	Ri 59b
G. disticha (L.) Haw.	14	Mr 34; Bn 69a, 71
as *G. lingua* Bgr.	14	Fg 26; Mr 34; Re 37a
G. disticha var. *comprucata*: see *G. conspurcata*		
G. elongata Bak.	14	Mr 34
G. ernestii-ruschii Dtr.	14	Ri 59b
G. excelsa Bak.: see *G. fuscopunctata*		
G. fasciata (S.D.) Haw.	14	KnM 43; Ri 59b; RiHMj 68
G. fuscopunctata Bak.	14	Ri 59d; RiMj 68
as *G. excelsa* Bak.	14	Fg 26
G. glabra Haw.	14	Re 37a; Ri 59b, 59d
G. gracilis Bak.		
as *G. gracilis* v. Poelln.	14	KnM 43
G. humilis v. Poelln.	14	Ri 59b; Bn 71
G. laetipuncta Haw.		
as *G. laetepuncta*	14	Re 37a
as *G. laetepunctata*	14	Ri 45
as *G. laetipunctata*	14	Ri 59d
G. laevis Haw.: see *G. angustifolia* var. *laevis*		
G. liliputana v. Poelln.	14	Sn 51a; ShM 65; Bn 71
[3 plants]	14	Ri 59b
G. lingua var. *conspurcata*: see *G. conspurcata*		
G. lingua Bgr.: see *G. disticha*		
G. lutzii v. Poelln.	14	Bn 69a, 71; CBn 77
G. maculata (Thunbg.) Haw.	14	Re 37a; Ri 59d; RiHMj 68; ShM 65
	28	St 37, 42
as *G. nigricans* var. *platyphylla* Bak.	14	Fg 26
as *G.* sp. aff. *maculata*	14	Gl 43
G. mollis Haw.	14	Mr 34; Ri 59d
G. neliana v. Poelln.	14	Re 37a; Bn 71
[2 plants from different localities]	14	Ri 59b
G. nigricans Haw.	14	Mr 34; Re 37a; St 37, 42; Ri 45, 59b, 59d; RiHMj 68; Bn 71
	28	KnM 43
G. nigricans var. *crassifolia* (Ait.) Haw.	28	Fg 26; Bn 77a
G. nigricans var. *platyphylla* Bak.: see *G. maculata*		
G. nitida (S.D.) Haw.	14	Re 37a; Ri 45, 59d
G. obtusa (S.D.) Haw.	14	Re 37a; Bn 71
G. obtusifolia (S.D.) Haw.	14	Re 37a; Ri 45, 59d; Bn 69a, 71
G. parvifolia Bak.	14	Bn 71
G. picta Bailey: see *G. picta* Haw.		
G. picta Haw.		
as *G. picta* Bailey	14	BbG 67
G. planifolia Bak.	14	Mr 34; Re 37a; St 37, 42; Ri 45, 59d; Bn 69a, 71; CBn 77

TABLE 5.2 Chromosome Numbers in *Gasteria* (*continued*):

Species	2*n*	Source
G. poellnitziana Jacobsen	14	Ri 59b; Bn 71
G. prolifera Lem.	14	Ge 35; Ja 47
G. pseudonigricans (S.D.) Haw.	14	Ri 59d; Bn 71
G. pulchra (Ait.) Haw.	14	Mr 34; Re 37a; KnM 43; Sn 51a; RiHMj 68
[4 plants]	14	Ri 59b
G. retata Haw.	14	Fg 26; Ri 59b, Bn 71
G. schweickerdtiana v. Poelln.	14	RiMj 67; Bn 69a, 71
[4 plants]	14	Ri 59b
G. spiralis Bak.	14	Bn 71
	28	Bn 71
G. subcarinata (S.D.) Haw.	14	Mr 34
G. subverrucosa (S.D.) Haw.	14	RiHMj 68
[2 plants]	14	Ri 59b
	28	Bn 69a, 71
G. sulcata (S.D.) Haw.	14	Mr 34; Ri 45, 59d; RiHMj 68
G. trigona Haw.	14	Re 37a; Ri 59d
G. trigona var. *kewensis* Bgr.	14	Bn 71
G. verrucosa (Mill.) Duv.	14	Bn 71; BbG 67
as *G. verrucosa* Haw.	14	Ta 24; Re 37a; KnM 43
[n. a.]	14	Mr 34; Ri 59b, 59d
G. verrucosa var. *asperrima* (S.D.) v. Poelln. [2 plants]	14	Ri 59d
G. verrucosa var. *latifolia* Haw.	14	St 37, 42; Bn 71
G. zeyheri (S.D.) Bak.	14	Ja 47; Ri 59d; RiHMj 68
[50 plants aff. *G. zeyheri*]	14	Ri 58, 59b
Species and Varieties Not Listed in Jacobsen (1954, 1970)		
G. cooperi	14	Fg 26
G. depressa	14	Ge 35
G. gigantea	14	RiHMj 68
[4 plants]	14	Ri 59b
G. glabra var. *major*	14	Bn 71
G. gracilis var. *minima*	14	St 42
as *Aloe gracilis* var. *minima*	14	St 37
G. huttoniae N. E. Br.	14	Bn 71
G. lignosum [2 plants]	14	Ri 59b
G. minima v. Poelln.	14	KnM 43; ShM 65
G. multipunctata	14	St 37, 42; RiHMj 68; Bn 71
G. nigricans var. *fasciata*	14	Ri 45
G. obscura	14	St 37, 42
G. parviflora	14	Ri 45, 59d
G. rotata	14	Fg 26
G. undulata	14	DK 57
G. vittata	14	Re 37a; St 37, 42
Unidentified Plants		
G. "aruhero"	14	St 37, 42
G. sp. "Gyu-zetu"	14	KnM 43
G. sp.	14	MtS 35
G. sp. [2 plants]	14	Re 37a
G. sp.	28	Re 37a
G. sp. [11 plants]	14	Sn 51b
G. sp.	14	Cl 58
G. sp. [48 plants]	14	Ri 59b

TABLE 5.2 Chromosome Numbers in *Gasteria* (*continued*):

Species	2*n*	Source
G. sp.	14	RiMj 67
G. sp. [from Hunt's Drift]	14	RiMj 67
G. sp. [another plant from Hunt's Drift]	14	RiHMj 68
G. sp. ["No. 2"]	14	Bn 71
G. spp. ["No. 3" and "No. 4"]	14	Bn 69a, 71
G. spp. [6 plants]	14	Bn 71
G. taxa [45 undesignated plants]	14	Bn 69b
Plants Regarded as Horticultural Forms		
G. amoena Hort. de Laet.	14	Bn 69a, 71
G. croucheri var. *spathulata* Hort. Kew	14	Fg 26
G. dicta N. E. Br. Hort. Kew	14	Ri 59d, 60b; Bn 69a, 71; CBn 77
G. nobilis Hort.	14	Bn 71; CBn 77
G. rurex Hort.	14	Sn 51a
G. sp. Hort.	14	Sn 51a
Putative Interspecific *Gasteria* Hybrids		
G. brevifolia X *G. nigricans*	14	Ri 45, 59d
G. brevifolia X *G. planifolia*	14	Ri 45, 59d
G. X*cheilophylla* Bak. (= *G. verrucosa* X *G. pulchra*)	14	Ta 24; Fg 26; Ge 35; Re 37a; St 37, 42; Ri 59b, 59d; Bn 71; CBn 77
	28	St 37, 42
G. lingua X *G. pulchra*	14	RiHMj 68
G. X*margaritifera* Bgr.	14	Ri 59d
G. planifolia X *G. nigricans*	14	Ri 45, 59d
G. planifolia X *G. sulcata*	14	Ri 59d
G. pulchra X *G. planifolia*	14	Ri 45, 59d
G. pulchra X *G. sulcata*	14	RiHMj 68
G. sulcata X *G. nigricans*	21	Ri 45, 48a; RiHMj 68
G. sulcata X *G. planifolia*	14	Ri 59d
G. verrucosa X *B. brevifolia*	14	Ri 45, 59d
G. verrucosa X *G. pulchra*	14	Ri 59d

TABLE 5.3 Chromosome Numbers in *Haworthia*

Species	2*n*	Source
Species Listed in Jacobsen (1954)		
H. altilinea Haw.	14	Ri 59b; Bn 71
H. altilinea var. *denticulata* (Haw.) v. Poelln. as *H. denticulata* Haw.	14	Re 37a
H. angustifolia Haw.	14	Ri 59b
H. angustifolia var. *albanensis* (Schoenl.) v. Poelln. [2 clones]	28	MjRi 67; Mj 68
H. angustifolia var. *liliputana* Uitew.	14	RiMj 65b, 66a
H. angustifolia var. nov.	14	Mj 68
H. arachnoidea (L.) Duv.	14	Mj 68
H. aristata Haw.	14	Sn 51a
as *Aloe aristata* Roem. & Schult.	14	Sn 51a

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. armstrongii v. Poelln.	14	Bn 71
	42	Pl 44, 46; Vv 59
	40	Mj 68
H. aspera Haw.: see *Astroloba aspera* [Table 5.4]		
H. asperiuscula Haw.	14	RiMj 65b, 66a; Bn 71
H. aff. *asperula* Haw.	14	Mj 68; Bn 71
H. atro-fusca G. G. Smith	14	RiMj 65b, 66a; MjRi 67
H. atrovirens Haw.: see *H. herbacea* (Mill.) Stearn.		
H. attenuata Haw.	14	Mr 34; Re 37a; St 37, 42; KnM 43; Sn 51a; Ri 59b; MkRi 61; RiMjH 66, 67; Mj 68
	28	KnM 43
H. attenuata var.?	14	MjRi 67
H. attenuata var. *britteniana* v. Poelln.	14	Bn 71
H. attenuata var. *caespitosa* (Bgr.) Farden	14	MkRi 61; RiMk 65; Bn 71
H. attenuata var. *minissima* Farden	14	Bn 71
H. baccata G. G. Smith	14	Mj 68
	28	RiMk 65
H. aff. *baccata*	14	MjRi 67
H. aff. *baccata* [2 different plants]	14, 28	Mj 68
H. batesiana Uitew. [2 plants]	14	RiMj 65b, 66a; Mj 68
H. blackbeardiana v. Poelln.	14	Bn 71
H. blackburniae f. nov.	14	Mj 68
H. bolusii Bak.	14	RiMj 65b, 66a; MjRi 67
H. broteriana Res.: see *H. sampaiana* f. *broteriana*		
H. browniana v. Poelln.	14	Mj 68; Bn 71; BnJ 77
H. carrissoi Res.	28	Pl 44, 46; RePl 46
H. chalwinii Marl. & Bgr.: see *H. reinwardtii* var. *chalwinii*		
H. chlorocantha Haw. [2 plants]	14	RiMj 65b, 66a
H. coarctata var. *coarctata* Haw.	14	Sn 51a
	28	Bn 71
	42	Re 37a, 38; KnM 43; Vv 59
as *H. coarctata* var. *haworthii* Res.	42	RePl 46; Sn 51a
as *H. coarctata* var. *haworthii* f. *major* Res.	42	Pl 46; Mj 68
H. coarctata var. *haworthii* f. *pseudo-coarctata* (v.P.) Res.: see *H. greenii* f. *pseudocoarctata*		
H. coarctata var. *krausii* Res.	42	Pl 44, 46; RePl 46; Sn 51a; Vv 59; Mj 68
H. coarctata var. *sampaiana* Res.: see *H. sampaiana*		
H. cooperi Bak.	14	Fg 26; Ri 59b; Mj 68; Bn 71
H. correcta v. Poelln.	14	Sn 51a
H. cuspidata Haw.	14	Re 37a; Sn 51a; Bn 71
H. cymbiformis (Haw.) Duv.	14	Sn 51a; Fi 59b
as *H. cymbiformis* Haw. [n. a.]	14	Re 37a; KnM 43; ShD 62
	14	Fg 26; St 37, 42

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. cymbiformis var. *angustata* v. Poelln.	14	Bn 71
H. cymbiformis var. *obesa* v. Poelln.	14	Mj 68
H. cymbiformis var. *obtusa* Bak.: see *H. obtusa*		
H. denticulata Haw.: see *H. altilinea* var. *denticulata*		
H. dielsiana v. Poelln.: see *H. obtusa* var. *dielsiana*		
H. eilyae var. *eilyae* v. Poelln.	14	RiMj 65b, 66a
as *H. eilyae* var. *poellnitziana* Res.	14	Pl 44, 46; Mj 68
H. eilyae var. *zantneriana* v. Poelln.	14	Pl 44, 46
H. aff. *eilyae*	14	Mj 68
H. fasciata (Willd.) Haw.	14	Re 37a; St 37, 42; KnM 43; ShM 65; MjRi 67; Mj 68
H. fasciata var. *vanstaadensis* v. Poelln.	14	RiMj 65b, 66a
H. ferox v. Poelln.	14	Mj 68
H. floribunda v. Poelln. var.?	14	Mj 68
H. fulva G. G. Smith	14	Sn 51a; RiMk 65; RiMj 68; Mj 68
H. gigas v. Poelln.: see *H. setata* var. *gigas*		
H. glabrata (S.D.) Bak.	14	Fg 26; Sn 51a
H. glabrata var. *perviridis* S.D.	14	Fg 26; Mj 68
H. glauca Bak.	28	Pl 46; Sn 51a; MjRi 67; RiMj 68; Mj 68; Bn 71
[small-leaved form]	28	RiMj 66b, 68; Mj 68
	29	Re 38
H. gracilis v. Poelln.	14	Bn 71
H. aff. *gracilis* v. Poelln.	14	Mj 68
H. greenii Bak. f. *greenii*	28	Vv 59; Mj 68; Bn 71
as *H. greenii* f. *bakeri* Res.	28	Pl 44, 46; RePl 46
H. greenii f *greenii* [isotype]	28	Mj 68
H. greenii f. *minor* Res.	28	Pl 44, 46; RePl 46; Mj 68
H. greenii f. *pseudocoarctata* (v.P.) Res.	28	RePl 46; Vv 59; Bn 71
as *H. coarctata* var. *haworthii* f. *pseudocoarctata* (v.P.) Res.	28	Pl 44, 46
H. greenii f. *pseudocoarctata*, "clone 6"	28	Mj 68
"clone 6"	30	RiMk 65
"clone 12"	28	RiMk 65; Mj 68
H. greenii aff. f. *pseudocoarctata*	28	Mj 68
H. greenii var. *silvicola* G. G. Smith	28	Sn 51a; RiMk 65; Mj 68
H. greenii f. nov.	28	Mj 68
H. haageana v. Poelln.	14	Bn 71
H. helmae v. Poelln. [type plant]	14	Mj 68
form	14	Mj 68
H. herbacea (Mill.) Stearn.		
as *H. atrovirens* Haw.	14	Sn 51a
as *H. pumila* Duv.	14	Sn 51a
H. herrei var. *depauperata* v. Poelln.	14	Sn 51a; Bn 71
	42	Pl 44, 46; Vv 59; Bn 71
[topotype]	42	Mj 68

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. herrei var. *herrei* v. Poelln.	14	Sn 51a
	42	Re 38
as *H. herrei* var. *poellnitzii* Res.	14	Pl 44, 46
[isotype]	14	Mj 68
H. hilliana v. Poelln.: see *H. umbraticola* var. *hilliana*		
H. hurlingii v. Poelln.	14	KnM 43; Ri 59b
H. hybrida (S.D.) Haw.	14	Fg 26; Re 37a; RiMk 65; MjRi 67; Mj 68; Bn 71; CBn 77
H. hybrida var.?	14	MkRi 61
H. icosiphylla Bak.	14	Sn 51a; Mj 68; Bn 69a, 71
H. jacobseniana v. Poelln.	14	Pl 44, 46; Vv 59
[isotype]	14	Mj 68
H. jonesiae v. Poelln.	14	Pl 44, 46; Mj 68
	42	Bn 71
H. jonesiae f.	35	Bn 71
H. kewensis v. Poelln.	14	Pl 44, 46; RePl 46; PlRe 49; Bn 69a, 71
H. lepida G. G. Smith	14	Sn 51a
H. limifolia Marl.	14	Re 37a
	28	Re 37a; RiMj 65a, 66a; Mj 68; Bn 71
H. limifolia var. *limifolia* Marl. as *H. limifolia* var. *marlotheana* Res.	28	Re 40, 49a
[2 plants]	21	Mj 68
H. limifolia var. *schuldteana* Res.	14	Re 40, 49a; ReV 48
	21	RiMjH 66, 69; MjRi 67; Mj 68
H. limifolia var. *stolonifera* f. *major* Res.	28	Mj 68
H. limifolia var. *stolonifera* f. *pimentellii* Res.	14	RiMk 65; MjRi 67
	21	Mj 68
H. limifolia var. *ubomboensis* (Verd.) G. G. Smith	14	Mj 68
H. limifolia var. nov. [plant #1]	21	MjRi 67
[plant #2]	23	RiMj 66b; MjRi 67
H. lisbonensis Res.	14	Pl 44, 46; RePl 46; Vv 59
H. sp. aff. *lisbonensis*	14	RiMk 65; Mj 68
H. longiana v. Poelln.	14	RiMj 65b, 66a; MjRi 67; Bn 71
H. sp. aff. *longiana*	14	RiMj 65b, 66a
H. maraisii v. Poelln. [2 clones]	14	Mj 68
H. margaritifera (L.) Haw.	14	Re 37a; St 37, 42; KnM 43; Mj 68
H. margaritifera var. *minima* (Ait.) Uitew. as *H. margaritifera* var. *granata* (Willd.) Bak.	14	St 37, 42; KnM 43
H. margaritifera var. *minima* subv. *polyphylla* (Haw.) Uitew.	14	Mj 68
H. margaritifera var. nov.	14	Mj 68
H. marumiana Uitew.	28	Bn 71
H. maughanii v. Poelln.	14	Re 37a; Sn 51a; Bn 71
[2 clones]	14	RiMj 66a; Mj 68
H. minima Bak.: see *H. tenera*		
H. mirabilis Haw.	14	St 37, 42; Mj 68
	42	RiMj 65a

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. aff. *morrisiae* v. Poelln.	14	RiMj 65b, 66a
H. mucronata Haw. as *H. mucronata* var. *altilinea* v. Poelln. [2 clones]	14	Mj 68
H. mucronata var. *polyphylla* (Bak.) v. Poelln.	14	Sn 51a
H. sp. aff. *mundula* G. G. Smith	14	Mj 68
H. musculina G. G. Smith	28	RiMk 65; Mj 68; Bn 71
[clonotype]	28	Mj 68
H. nigra Bak.	14	Bn 69a, 71
H. nigra var. *elongata* (v.P.) Uitew.	21	Mj 68
H. nigra var. *schmidtiana* (v.P.) Uitew. as *H. schmidtiana* v. Poelln.	21	Bn 71
H. notabilis v. Poelln.	14	Mj 68
H. obtusa Haw. emend. Uitew.		
as *H. cymbiformis* var. *obtusa* Bak.	14	Ta 25b; Mj 68
as *H. obtusa* Haw.	14	Bn 71
H. obtusa var. *columnaris* Uitew.	14	Bn 71
H. obtusa var. *dielsiana* (v.P.) Uitew. as *H. dielsiana* v. Poelln.	14	Bn 71
H. obtusa var. *pilifera* (Bak.) Uitew.	14	Bn 71
as *H. pilifera* Bak.	14	Re 37a
H. otzenii G. G. Smith	14	Bn 71
H. pallida Haw.	14	Bn 71
H. papillosa (S.D.) Haw.	14	Re 37a; ShM 65; Mj 68; Bn 71
H. paradoxa v. Poelln.	14	Mj 68
H. peacockii Bak.	14	Mj 68
H. pellucens Haw.: see *H. translucens*		
H. perplexa v. Poelln.	14	Sn 51a
H. picta v. Poelln.	14	RiMj 65b, 66a
H. pilifera Bak.: see *H. obtusa* var. *pilifera*		
H. planifolia Haw.	14	Mr 34; Re 37a; KnM 43; Sn 51a; ShM 65; RiMk 65; Mj 68; Bn 71
H. planifolia var. *planifolia* Triebn. & v. Poelln.	14	MkRi 61; RiMj 66a
H. planifolia var. *planifolia* f. *agavoides* Triebn. & v. Poelln.	14	RiMj 65b, 66a
H. planifolia aff. var. *setulifera* v. Poelln.	14	Mj 68
H. planifolia var. nov.	14	ReMj 65b, 66a
H. poellnitziana Uitew.	14	Bn 71
H. aff. *poellnitziana* Uitew.	14	Mj 68
H. aff. *poellnitziana* var. nov. [2 plants]	14	Mj 68
H. aff. *pseudogranulata* v. Poelln.	14	Mj 68
H. pseudotortuosa S.D.: see *H. viscosa* var. *pseudotortuosa*		
H. pumila Duv.: see *H. herbacea*		
H. radula (Jacq.) Haw.	14	Fg 26; Re 37a; St 37, 42; KnM 43; ShD 62; Bn 71
H. ramosa G. G. Smith	14	Sn 51a; RiMj 66a; MjRi 67; Bn 71
H. recurva Haw.	14	Fg 26
	28	Mj 68
H. reinwardtii (S.D.) var. *adelaidensis* v. Poelln.	14	Pl 44, 46; RePl 46; Sn 52; Bn 71
[topotype]	28	Mj 68

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. reinwardtii aff. var. *adelaidensis*	14	Mj 68
H. reinwardtii var. *archibaldiae* v. Poelln.	14	Bn 69a, 71
	21	Pl 44, 46; RePl 46; Sn 52; Vv 59
	28	Re 38
H. reinwardtii var. *brevicula* G. G. Smith	14	Sn 51a
H. reinwardtii var. *chalumnensis* G. G. Smith	21	Sn 51a; RiMjH 67; Mj 68; Bn 74 (47 plants)
	28	Bn 74 (98 plants)
H. reinwardtii var. *chalwinii* (Marl. & Bgr.) Res.	28	Pl 46; RePl 46; Sn 52; RiMk 65; Bn 71
[2 clones]	28	Mj 68
	26	RiMk 62
as *H. chalwinii* Marl. & Bgr.	28	Re 37a, 38
H. reinwardtii aff. var. *chalwinii*	28	Mj 68
H. reinwardtii var. *committeesensis* G. G. Smith	28	Sn 51a
H. reinwardtii var. *conspicua* v. Poelln.	28	Re 38; Pl 46a; RePl 46; PlRe 49; Mj 68; Bn 71
H. reinwardtii var. *diminuta* G. G. Smith	28	Mj 68
H. reinwardtii var. *fallax* v. Poelln.	14	Mj 68
	28	Pl 44, 46; RePl 46; Vv 59; Mj 68; Bn 71
H. reinwardtii var. *grandicula* G. G. Smith	14	Sn 51a; Bn 71
H. reinwardtii var. *huntsdriftensis* G. G. Smith	28	Sn 51a; RiMk 65; Mj 68
H. reinwardtii var. *kaffirdriftensis* G. G. Smith	14	Sn 51a; Mj 68
H. reinwardtii var. *major* Bak.	14	Pl 44, 46; RePl 46
	28	Re 38
as *H. reinwardtii* var. *pulchra* v. Poelln.	28	Re 38
H. reinwardtii var. *minor* Bak.	14	Pl 44, 46; RePl 46; Vv 59; Bn 69b, 71
H. reinwardtii aff. var. *minor* Bak.	14	Mj 68
	28	Mj 68
H. reinwardtii var. *olivacea* G. G. Smith	14	Mj 68
H. reinwardtii aff. var. *olivacea* [2 plants]	14	RiMk 65; Mj 68
H. reinwardtii var. *peddiensis* G. G. Smith	21	Sn 51a
H. reinwardtii var. *reinwardtii* Haw.	14	Vv 59
as *H. reinwardtii*	14	ShD 62
[7 plants]	14	Ri 59b; Mj 68
	28	Re 37a, 38; Vv 59; Bn 71
	42*	St 37, 42
as *H. reinwardtii* var. *haworthii*	14	RiMk 65; Mj 68
	28	Pl 44, 46; RePl 46

*Pinto-Lopes thinks this is probably *H. coarctata*.

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. reinwardtii var. *riebeekensis* G. G. Smith	14	Sn 51a
H. reinwardtii var. *tenuis* G. G. Smith	14	MkRi 61; RiMk 65; Mj 68
H. reinwardtii var. *triebneri* Res.	14	Pl 44, 46a; RePl 46
H. reinwardtii aff. var. *triebneri*	14	Mj 68
H. reinwardtii var. *valida* G. G. Smith	28	Sn 51a; RiMk 65; Mj 68
H. reinwardtii var. nov.	14	Mj 68
[3 other plants]	28	RiMk 65; Mj 68
	42	RiMj 66a; Mj 68
H. resendeana v. Poelln.	21	Re 38; Pl 46a; RePl 46; Sn 52; RiMj 65a; Bn 71
[clonotype]	21	RiMj 66a
[small-leaved form]	21	Mj 68
H. reticulata Haw.	14	Re 37a; MjRi 67
H. retusa (L.) Haw.	14	Re 37a; St 37, 42; KnM 43; Mj 68
H. retusa aff. var. *densiflora* G. G. Smith	14	Mj 68
H. revendettii Uitew.	35	Pl 44, 46; RePl 46; RiMj 65a, 66a; Bn 71
H. rigida (Lam.) Haw.	14	Fg 26; Re 37a; Mj 68; Bn 71
H. rubrobrunea v. Poelln.	35	Pl 44, 46; Sn 51a
[clonotype]	14	Mj 68
H. rugosa (S.D.) Bak.	14	Re 37a
H. ryderiana v. Poelln.	14	Ri 59b; Bn 71
H. sampaiana Res.	21	Bn 69a, 71
[clonotype]	35	Mj 68
	36	Pl 44, 46; RePl 46; Sn 51a; Vv 59
as *H. coarctata* var. *sampaiana* Res.	39 or 40	Re 38
H. sampaiana f. *broteriana* (Res.) Res. & Pinto-Lopes	35	RePl 46; Sn 51a; Vv 59; MjRi 67
as *H. broteriana* Res.	35	Pl 44, 46
H. sp. aff. *sampaiana*	35	Mj 68
H. schmidtiana v. Poelln.: see *H. nigra* var. *schmidtiana* Uitew.		
H. schuldtiana var. *erecta* Triebn. & v. Poelln.	14	RiMj 65b, 66a
H. schuldtiana var. *sublaevis* v. Poelln.	14	Mj 68
H. schuldtiana var. *whitesloaneana* v. Poelln.	14	Mj 68
H. schuldtiana var. nov.	14	Mj 68
H. semiglabrata Haw.	14	Re 37a; Mj 68
H. sessiliflora Bak.	14	Bn 71
H. setata Haw.	14	Re 37a
H. setata var. *gigas* v. Poelln. as *H. gigas* v. Poelln.	14	Re 37a
H. setata var. *xyphiophylla* (Bak.) v. Poelln.	14	Mj 68
H. sordida var. *agavoides* Zant. & v. Poelln.	14	Bn 71
H. spiralis: see *Astroloba pentagona* [Table 5.4]		
H. starkiana v. Poelln.	14	Mj 68; Bn 71

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. subattenuata (S.D.) Bak.	14	St 37, 42; Mj 68
H. subfasciata (S.D.) Bak.	28?	Fg 26
	14	ChS 76
H. subfasciata var.?	14	Mj 68
H. aff. *sublimpidula* v. Poelln.	14	Mj 68
H. subregularis Bak.	14	Sn 51a; Bn 71
H. subrigida Bak.: see *H. tortuosa* var. *pseudorigida*		
H. subulata (S.D.) Bak.	14	Re 37a; Bn 71
H. tenera (Bak.) v. Poelln. as *H. minima* Bak.	14	Re 37a
H. tessellata Haw.	14	Fg 26; Re 37a; ShD 62
	28	Fg 26; Re 37a; KnM 43
	35	KnM 43; RiMj 65a, 66a
	42	Re 40; St 37, 42; KnM 43; Bn 71
	56	Sn 51a
H. tessellata var. *coriacea* f. *brevior* Res. & v. Poelln.	58?	Vv 49
H. tessellata var. *coriacea* f. *longior* Res. & v. Poelln.	61?	Vv 49
H. tessellata var. *engleri* (Dtr.) v. Poelln.	28	Re 40; RiMj 66a; Mj 68
	40	RiMk 62
H. tessellata var. *inflexa* Bak.	14	RiMj 65b, 66a
	28	Re 40
H. tessellata var. *luisieri* Res. & v. Poelln.	56	ReV 59
	63	Vv 49
H. tessellata var. *minutissima* (v.P.) Viveiros	28	Vv 49
H. tessellata var. *obesa* Res. & v. Poelln.	56	Vv 49†
H. tessellata var. *palhinhae* Res. & v. Poelln.	56?	Vv 49
H. tessellata var. *parva* (Haw.) Bak.	28	Fg 26
H. tessellata var. *simplex* Res. & v. Poelln.	28	Vv 49†
H. tessellata var. *stephaneana* Res. & v. Poelln.	42	Vv 49†
H. tessellata aff. var. *stephaneana* (from Kiel)	42	Vv 49
H. tessellata var. *velutina* Res. & v. Poelln.	56?	Vv 49
H. tessellata var. nov.	28	Re 40
	42	Re 40
[2 plants]	56	Re 40
H. tessellata var.?	28	Mj 68
	42	RiMj 66a; MjRi 67; Mj 68
H. tisleyi Bak.	14	Bn 71
[topotype]	14	Mj 68
H. torquata Haw.: see *H. viscosa* var. *torquata*		
H. tortuosa Haw.	14	Mr 34; MkRi 61; RiMk 65
[type plant]	14	Mj 68

†Also in Resende (1940a) according to Viveiros (1949).

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. tortuosa var. *curta* Haw. [2 plants]	14	MkRi 61; RiMk 65; RiMj 65b, 66a, 67; Mj 68; Bn 71
H. tortuosa var. *major* (S.D.) Bgr.	14	MkRi 61; RiMk 65
H. tortuosa var. *pseudorigida* (S.D.) Bgr.	14	Re 37a; MkRi 61; RiMk 65; Mj 68; Bn 69a, 71
as *H. subrigida* Bak.	14	St 37, 42
H. tortuosa var. *tortella* (Haw.) Bak.	14	MkRi 61; RiMk 65; Mj 68
[2 plants]	14	MkRi 61; RiMk 65; Mj 68
H. tortuosa var.?	14	Sn 51a
H. translucens Haw. [2 plants]	14	Mj 68
as *H. pellucens* Haw.	14	Re 37a
H. triebneriana v. Poelln. var. nov.	14	Mj 68
H. truncata Schoenl.	14	Re 37a; Sn 51a; Mj 68
H. truncata f. *normalis* v. Poelln.	14	Mj 68
H. truncata Schoenl. f. *tenuis* v. Poelln.	14	Bn 71
H. tuberculata var. *acuminata* v. Poelln.	14	Mj 68
H. tuberculata var. *angustata* v. Poelln.	14	Mj 68
H. tuberculata var. *subexpansa* v. Poelln.	14	Mj 68
H. turgida Haw.	14	Re 37a; Sn 51a
H. turgida var. *pallidifolia* G. G. Smith	14	Mj 68
H. uitewaaliana v. Poelln.	14	Ri 59b; Bn 71
H. umbraticola v. Poelln.	14	Bn 71
H. umbraticola var. *hilliana* v. Poelln. as *H. hilliana* v. Poelln.	14	Sn 51a
H. variegata L. Bol. [small form]	14	Mj 68
[large and intermediate forms]	14	RiMj 65b, 66a, 67
H. venosa (Lam.) Haw.	14	Re 37a
H. viscosa (L.) Haw.	14	Re 37a; KnM 43; Ja 47; Sn 51a; Bn 71
H. viscosa var. *pseudotortuosa* (S.D.) Bak. as *H. pseudotortuosa* S.D.	28	Fg 26
H. viscosa var. *quaggaensis* G. G. Smith	14	Mj 68
H. viscosa var. *torquata* (Haw.) Bak. as *H. torquata* Haw.	14	St 37, 42a
H. viscosa var. nov.	14	Mj 68
H. vittata Bak.	14	Bn 71
H. zantneriana v. Poelln.	14	Mj 68
Horticultural Varieties		
H. fasciata var. *aureostriata* Hort.	14	KnM 43
H. krausiana Hort. Haage & Schmidt	14	Sn 51a
H. krausii Hort. Haage & Schmidt	14	RiMk 65
H. margaritifera var. *aureovariegata* Hort.	14	KnM 43
H. walmsley Hort.	35	RiMk 65; Mj 68
Hybrids or Putative Hybrids		
H. cassytha Bak. (= *H. lisbonensis* x *H. tortuosa*)	14	RePl 46; Bn 71

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. coarctatoides Res. & A. Viveiros	42	ReV 48; Sn 51a; Mj 68
H. herrei v. Poelln. X *H. reinwardtii* var. *minor* Bak.	14	Pl 46
H. jacobseniana v. Poelln. X *H. reinwardtii* var. *minor* Bak.	14	Pl 46
H. "kotobuki" X *H. retusa*	14	St 37
H. limifolia X *H.* sp., Coarctatae sec.	21, 35	ReV 48
H. manda hybrid	14	Mj 68
H. X*mantelii* (= *H. cuspidata* Haw. X *H. truncata* Hort.)	28	Mj 68
H. X*mantelii* (= *H. truncata* Hort. X *H. cuspidata* Haw.)	14	Mj 68
H. maughanii X *H. angustifolia*	14	Mj 68
H. maughanii v. Poelln. X *H. pilifera* Bak.	14	Bn 71
H. retusa X *H. cymbiformis*	14	St 42
H. setata Haw. X *H. obtusa* var. *prolifera*	14	Mj 68
H. tessellata var. *minutissima* X *H. tessellata* var.?	35	Vv 49
H. tessellata var. *palhinhae* X *H. tessellata* var.?	49	Vv 49
H. truncata X *H. setata* Haw.	14	Mj 68

Species Not Listed in Jacobsen (1954, 1970)

H. angusta nom. nud.	14	Sn 51a
H. asperula var. *albipapillosa* nom. nud.	14	Mj 68
H. beanii G. G. Smith	14	Sn 51a; RiMj 66a, 67
H. beanii var. *minor* G. G. Smith	14	Sn 51a; RiMj 66a
H. bicarneata nom. nud.	21	MjRi 67
H. caudata nom. nud.	14	St 37, 42
H. cuspi Haw. [*H. cuspidata* Haw.?]	14	Ru 64
H. fasciata "hakutei"	14	St 37
H. glabrata var. *semiglabrata* nom. nud.	14	RiMj 65
H. helos nom. nud.	14	St 37, 42
H. "kinzyo"	14	St 37
H. laevis nom. nud.	14	Fg 26
H. longibracteata G. G. Smith	14	Bn 71
H. longifolia nom. nud.	14	RiMj 65b, 66a
H. margaritifera var. *albo-variegata*	14	St 42
H. monticola nom. nud.	14	RiMj 65b, 66a, 67; Mj 68
H. nigra var. *ryneveldtii***	14	RiMj 65b, 66a
H. radula var. *variegata*	14	St 42
H. rubicunda	14	Mj 68
H. rubriflora Bolus	14	Re 37a
H. skinneri (Bgr.) Res.	14	Mj 68
H. speciosa nom. nud.	14	Mj 68
H. syringoidea?	14	Sn 51a
H. tenera var. *minima* v. Poelln.	14	Mj 68
H. tenuis	14	Sn 52
H. tessellata var. *parva* subv. *major*	28	RiMj 65a, 66a; MjRi 67

**This variety is not listed in Jacobsen (1954, 1970). *H. ryneveldiae* v. Poelln. is given as a synonym for *H. nigra* (Haw.) Bak.

TABLE 5.3 Chromosome Numbers in *Haworthia* (*continued*):

Species	2*n*	Source
H. sp. [1 plant]	14	MtS 35
H. sp. [4 plants]	14	Re 37a
H. sp. nov.	14	Mj 68
H. sp. [35 new determinations]	14	Sn 51b
[2 new determinations]	21	Sn 51b
[5 new determinations]	28	Sn 51b
[2 new determinations]	35	Sn 51b
[3 new determinations]	42	Sn 51b
[1 new determination]	56	Sn 51b
H. sp., Coarctatae sec.	14	RiMj 65b, 66a; Mj 68
[3 plants]	28	Mj 68
	42	Mj 68
H. sp. of Laetevirentes sec.	14	Mj 68
H. sp. nov., Obtusatae sec.	14	Mj 68
H. sp. nov., Retusae sec. [4 plants]	14	RiMj 65b; Mj 68
H. sp., Rigidae sec.	14	MkRi 61; RiMj 65b, 66a
[5 plants]	14	Mj 68
H. sp. nov., Tessellatae sec. [2 plants]	28	RiMj 66a; Mj 68
H. sp., Tortuosae sec.	14	MkRi 61; RiMk 65b
H. sp. between *H. chlorocantha* and *H. angustifolia*	14	Mj 68
Haworthia [57 taxa]	14	Bn 69b

TABLE 5.4 Chromosome Numbers in Other Genera

Species	2*n*	Source
Astroloba Uitew. (*Apicra* Haw.)		
As. aspera (Willd.) Uitew.	14	Mj 68
as *Apicra aspera* (Willd.) Haw.	14	Fg 26
as *Haworthia aspera* Haw.	14	Sn 51a
As. bicarinata (Haw.) Uitew.		
as *Apicra bicarinata* Haw.	21	Re 37a
As. congesta (S.D.) Uitew.	14	Mj 68
as *Apicra congesta* (S.D.) Bak.	18?	Mr 34
As. congesta var. *deltoidea*	14	Mj 68
As. deltoidea (Hook. f.) Uitew.		
as *Apicra deltoidea* Bak.	14	Fg 26; Re 37a
As. dodsoniana Uitew.	14	Mj 68
As. egregia (v. Poelln.) Uitew.	14	Bn 71
As. foliolosa (Willd.) Uitew.	14	Mj 68; Bn 71
as *Apicra foliolosa* (Willd.) Haw.	14	Re 37a
As. herrei Uitew.	14	Mj 68; Bn 71
As. pentagona (Haw.) Uitew.	21	Bn 71
as *Apicra pentagona* (Haw.) Willd.	28	Re 37a
as *Haworthia spiralis*	14	St 37, 42
As. pentagona var. *spiralis* (Haw.) Uitew.		
as *Apicra pentagona spiralis*	28	Fg 26
As. pentagona var. *spirella* (Haw.) Uitew.	21, 28	Mj 68
As. skinneri (Bgr.) Uitew.	14	Mj 68
As. sp. aff. *skinneri*	14	Mj 68
As. sp.	14	Ge 35; Su 36; Ri 59; Mj 68
Astroloba [6 taxa]	14	Bn 69b, 71

TABLE 5.4 Chromosome Numbers in Other Genera (*continued*):

Species	2*n*	Source
	Poellnitzia Uitew.	
P. rubriflora (L. Bol.) Uitew.	14	Vv 49; Mj 68
as *Apicra rubriflora* L. Bol.	14	Re 37a
	Chamaealoe Bgr.	
C. africana (Haw.) Bgr.	14	Vv 49; Bn 69a, 71
Chamaealoe [1 taxon]	14	Bn 69b
	Lomatophyllum Willd.	
L. orientale H. Perr.	14	Re 37a
L. purpureum (Lam.) Th. Dur.	14	Bn 71
Lomatophyllum [1 taxon]	14	Bn 69b
	Leptaloe Stapf	
L. albida Stapf	14	Muf 45
L. saundersii Reyn.	14	Muf 45

Chromosome studies have not been reported for *Chortolirion* or *Guillauminia* to date.

TABLE 5.5 Chromosome Numbers in Intergeneric Hybrids

Species	2*n*	Source
	Hybrids between *Aloe* and *Gasteria*	
Gastrolea X*bedinghausii* (Radl.) E. Walth		
as *Aloe* X*bedinghausii* Radl.	14	Re 37a
Gastrolea X*beguinii* (Radl.) E. Walth		
as *Aloe* X*beguinii* Hort.	14	Re 37a; ShM 65
Gastrolea X*lapaixii* (Radl.) Jacobs.		
as *Aloe* X*lapaixii* Radl.	14	Re 37a; Bn 71
Gastrolea X*novotnyi* (Radl.) E. Walth.	20	Bn 69a, 71
Gastrolea X*rebutii* (Bgr.) E. Walth.		
as *Gasteria rebutii* Hort.	14	Bn 69a, 71
Gastrolea X*smaragdina* (Hort. Bgr.) E. Walth		
as aff. *smaragdina*	14	Bn 69a, 71
Gastrolea [2 taxa]	14	Bn 69b
Gasteria carinata Haw. X *Aloe dorotheae* Bgr.	14	CBn 77
Gasteria cheilophylla X *Aloe variegata* L.	14	CBn 77
Gasteria dicta N.E. Br. X *Aloe dawei* Bgr.	21	CBn 77
Gasteria "Gyu-zetu" X *Aloe variegata*	14, 28	St 37, 42
	14	SiSt 40
Gasteria lutzii v. Poelln. X *Aloe aristata* Haw.	14	CBn 77
Gasteria lutzii v. Poelln. X *Aloe tenuior* var. *rubriflora* Reyn.	14	CBn 77
Gasteria planifolia Bak. X *Aloe dorotheae* Bgr.	14	CBn 77
Gasteria verrucosa X *Aloe variegata*	14	KnM 43
Gasteria sp. X *Aloe* sp. [2 different plants]	14	Ri 47, 48b, 50
Aloe variegata X *Gasteria* "Gyu-zetu"	14	SiSt 40; St 42
Aloe variegata X *Gasteria* sp. "Seiryuto"(?)	14	KnM 43
Aloe variegata X *Gasteria verrucosa*	14	KnM 43
Aloe variegata X *Gasteria verrucosa* var. *latifolia*	14	St 37,42; SiSt 40

TABLE 5.5 Chromosome Numbers in Intergeneric Hybrids (*cont.*):

Species	2*n*	Source
Hybrid between *Astroloba* and *Gasteria*		
Gasteria X*apricoides* Bak.	ca. 14	Fg 26
Hybrids between *Gasteria* and *Haworthia*		
Gasterhaworthia X*bayfieldii* (Salm.) Rowley	14	Ro 54; Bn 71
as *Gasteria bayfieldii* Bak.	14	Sn 51a
Gasterhaworthia X*holtzei* (Radl.) Guill. as *Gasteria holtzei*	14	Fg 26
Gasterhaworthia Xsp. as *Haworthia* sp. X *Gasteria* sp.	14	KnM 43; Mj 68
Gasterhaworthia [1 taxon]	14	Bn 69b
Haworthia tessellata X *Gasteria* sp.	14	Mj 68
Haworthia hybrida Haw. X *Gasteria notabilis* Hort.	14	CBn 77

Chapter Six

POLYPLOIDY

Soon after cytologists began to observe chromosome numbers in plants, they found some plants with a chromosome number that was a multiple of the haploid number found in other members of the species but a multiple higher than the normal diploid number. For example, the brilliant plant physiologist and geneticist Hugo de Vries had advanced his Mutationstheorie in 1901 and listed among his mutants of *Oenothera lamarckiana* a *gigas,* or giant type, and a *semi-gigas,* a half-giant. A few years later Lutz (1907) and Gates (1909) examined the chromosomes and found that if the normal *O. lamarckiana* plant had $2n = 14$ chromosomes, in *gigas* the somatic or sporophytic number was 28 and in *semi-gigas* it was 21, making these plants a tetraploid and a triploid respectively. Within a dozen years a number of other polyploid plants were found in other species and genera.

Polyploids in plants can be divided into two basic types — autopolyploids and allopolyploids. Some subtypes such as segmental allopolyploids and autoalloploids are also found, but they are variations or combinations of the two main types. If the chromosomes of the diploid species *A* were doubled, an autotetraploid would be produced; but if species *A* and the diploid species *B* were crossed to produce a sterile diploid hybrid and the chromosomes in this hybrid were doubled, an allotetraploid would arise. This sounds simple and clear-cut, but actually very little in nature follows man-made rules precisely. It would have been simple if rules had been laid down and nature fitted into them, but nature came first, and it is man's job to try to find patterns in nature that can be expressed in rules. Unfortunately nature did not develop in such a way that nice, clear-cut patterns can usually be established. Actually the difference between an autopolyploid and an allopolyploid is often not clear. A typical autotetraploid is somewhat (perhaps 20-30%) sterile and ideally has many multivalent chromosome configurations in the first meiotic metaphase. Theoretically they should all be quadrivalents, but occasional failure of pairing or chiasma formation results in trivalents and univalents or bivalents.

The allopolyploid behaves ideally as a diploid. Thus an allotetraploid could be considered an amphidiploid or a "double diploid," and all the chromosomes should be arranged in bivalents. Actually the further apart two species are in a phylogenetic sense, the more frequently they

should form only bivalents; but if two species were so unrelated that their chromosomes would have no segments in common, they might not even be close enough to hybridize and form a viable hybrid. Normally the two sets of chromosomes might have a number of common segments; if so, they would form some quadrivalents and might tend to be confused with the autotetraploids. It is difficult, therefore, to be absolutely sure that a given plant is an autopolyploid unless one knows its origin, because the possession of quadrivalents is not infallible evidence.

In the higher plants polyploidy, especially allopolyploidy, is a rather common method of evolution; perhaps as many as one-third of the angiosperms are polyploids. Allopolyploidy is a mechanism by which a new species can be formed suddenly, and in that sense it may be considered a factor in plant evolution. The new species will usually combine characters of the two parental species and because of the chromosome number will usually be reproductively isolated from the two parental types. However, hybridization followed by chromosome doubling does not usually contribute anything fundamentally new; it merely represents a reshuffling of traits already present, so it actually contributes little to progressive evolution.

As was pointed out in Chapter 4, the normal karyotype of a diploid plant of the Aloineae consists of eight long and six short chromosomes (Fig. 6.1; see also Fig. 4.1). Apparently the first polyploids reported in the Aloineae were listed by Ferguson (1926), who included eight in her Table I. Ferguson's *Gasteria nigricans crassifolia* apparently was a tetraploid, although she never obtained an exact count of the short chromosomes. She found more polyploids in *Haworthia* than in the other genera, a fact that subsequent studies by many other people have confirmed. She found 28(?) in *H. subfasciata* and 28 in two *tessellata* plants of somewhat different origins. She made meiotic studies of some plants, and in the haploid set of *H. tessellata parva* found eight long and six short chromosomes. Eight long chromosomes were found in *H. pseudotortuosa,* but the short ones could not be counted. There was one tetraploid in *Astroloba — As. pentagona spiralis.*

According to Table I of Ferguson (1926), *Aloe ciliaris* had more than 45 chromosomes; soon after her paper appeared, Gioelli (1930) reported more than 50 chromosomes in that species. Resende (1937a, 1938), Schnarf and Wunderlich (1939), Müller (1941, 1945), Snoad (1951a), Resende and Amaral (1956), Riley (1958, 1959a), Brinckmann (1960), Sharma and Mallick (1965), and Brandham (1971) gave 42 as the somatic chromosome number of *Aloe ciliaris;* thus Ferguson's and Gioelli's plants must have been aneuploids (a hyperhexaploid and a hyperheptaploid). On the other hand, Ferguson's data and especially Gioelli's were possibly inaccurate.

More recent determinations of chromosome numbers include several polyploids in *Aloe*. According to Sharma and Mallick (1965), *A. humilis* is a triploid. Brandham (1971) reported a somatic number of 28 chromosomes in *A. dawei, A. elgonica, A. jacksonii,* and *A.* sp. "juvenna."

Early in the development of Aloineae cytology Sato (1937) came across several new polyploid plants, among which were *Gasteria maculata* ($2n = 28$) and *Haworthia rein-*

6.1 Camera lucida drawing of chromosomes of the diploid *Haworthia starkiana*.

wardtii (2*n* = 42). He also found a hexaploid plant of *H. tessellata* and a tetraploid hybrid between a *Gasteria* plant (*G.* "Gyu-zetu") and *Aloe variegata*. About the same time, Resende (1937a) examined the chromosomes of a number of the Aloineae. Of 84 species, varieties, and hybrids of *Aloe* only *A. ciliaris* Haw. had other than the usual diploid number of chromosomes; it was a hexaploid. Three plants that he placed under *Aloe* but more properly are placed in *Gastrolea,* a hybrid genus, were also diploids. Of 21 *Gasteria* plants an unidentified species was a tetraploid and the rest were diploids. *Haworthia* and *Astroloba* had a higher percentage of polyploids. Thirty-six *Haworthias* were studied: *H. reinwardtii* and *H. reinwardtii* var. *chalwinii* (Marl. & Bgr.) Res. (as *H. Chalwinii* Marl. & Bgr.) were tetraploids; *H. coarctata* Haw. was a hexaploid; and both *H. limifolia* Marl. and *H. tessellata* Haw. had both diploid and tetraploid forms. This study apparently was the first of *Lomatophyllum,* the "aloe" of Madagascar; *L. orientale* Perrier was a diploid. *Astroloba pentagona* Wild. was a tetraploid, *As. pentagona* var. *spirella* Bak. was a triploid, and four other species were diploids.

The percentage of polyploid species in the different genera has been of interest, and several cytologists have compiled tables. The first to do so was Resende (1940a). According to him the percentage of polyploid forms is 2.248 in *Aloe,* 25.0 in *Astroloba,* 10.5 in *Gasteria,* 28.8 in *Haworthia,* and 13.2 for all the Aloineae studied. Such tables undoubtedly are useful in a very general way (certainly the frequency of polyploids is higher in *Haworthia* than it is in *Aloe* and *Gasteria*), but otherwise they do not mean much because they do not cover enough plants. To state that a given species is diploid or tetraploid on the basis of one or two individuals can lead to great inaccuracies; in an organism that reproduces vegetatively, as do the Aloineae, several reports even from botanical gardens widely scattered geographically might actually concern different offshoots from the same clone. Resende (1940a) found diploid, tetraploid, hexaploid, and octoploid forms of *H. tessellata,* but they could not be distinguished morphologically. The many polyploids in *Haworthia* are by no means evenly distributed among the various sections; they are most numerous in the Coarctatae section (which includes *H. reinwardtii* and *H. coarctata*), the Limifoliae section (which includes *H. limifolia*), and the Tessellatae section (which includes *H. tessellata*).

Resende and his students (Pinto-Lopes 1946) have noted the situation in the Coarctatae section where the species make "a complete, natural, polyploid series formed of the terms 2*x*, 3*x*, 4*x*, 5*x*, 6*x*." The percentage of polyploids within the Coarctatae section is 63.3; the percentage in the genus *Haworthia* as a whole, according to Pinto-Lopes (1946), is 31.2. Other species of the section also are polyploids. *H. sampaiana* f. *broteriana* is a pentaploid (Resende and Pinto-Lopes 1946) which was growing well in the botanical garden in Lisbon, Portugal. It bore fruit there throughout the whole summer, and the seeds germinated well. *H. carrissoi,* a tetraploid, and *H. revendettii,* a pentaploid, also grew well and produced fruit and viable seeds in Lisbon.

A species of a section that has a high percentage of polyploidy is *H. reinwardtii* with its many varieties. *H. reinwardtii* vars. *adelaidensis, triebneri, minor,* and *major* are diploids, according to Pinto-Lopes (1946), and var. *archibaldiae* is a triploid. This list, however, illustrates how misleading it can be to publish a chromosome number based on one or two specimens, because subsequently Majumdar (1968) found a tetraploid plant of var. *adelaidensis*. Resende's (1938) report of 28 sporophytic chromosomes in var. *major* has been ignored, and his report (Resende 1938) that var. *archibaldiae* is a tetraploid is discredited by Pinto-Lopes (1946), who states: "From observation of our preparation obtained by means of smears of root-tips we assert that it is triploid, since we established the existence of 12 large chromosomes and 9 small." *H. reinwardtii* vars. *conspicua, chalwinii, fallax,* and *reinwardtii* (published as var. *typica* [= *major* Bak.] v.P. in Resende [1938]) were listed as tetraploids. Since then Majumdar (1968) has found a plant identified as var. *fallax* that is a diploid, and several people (Riley 1959b; Sharma and Datta 1962; Majumdar 1968) have found diploid plants of var. *reinwardtii*. Sato (1937, 1942) lists *H. reinwardtii* var. *reinwardtii* (as *H. Reinwardtii*) as a hexaploid. Pinto-Lopes (1946) did not find any hexaploid plant of that variety and maintains that the variety is tetraploid only. He dismisses Sato's report by stating that Sato was probably dealing with a plant of *H. coarctata;* to support his idea he points out that in the Lisbon Botanical Garden "all the specimens of *Haworthia coarctata* f. *major* are found to be classified under *Haworthia Reinwardtii*." The present authors fail to see that the confusion of species in Lisbon would necessarily result in a similar confusion in Japan. Pinto-Lopes felt that "it is almost certain that Sato also used specimens of *Haw. coarctata* which also in his garden were considered as belonging to the species *Haw. Reinwardtii,*" but offered no evidence in support of his certainty.

Pinto-Lopes and some of his colleagues seemed to feel that all the plants of a given species or variety must have the same chromosome number, which eliminates the possibility of polyploidy within a species or variety. In any genus where identification of species and varieties is so difficult, it is almost impossible either to establish or to refute such a claim. Resende and Viveiros (1948) found a plant in their collection that seemed to have the color and general appearance of the leaves of *H. coarctata* var. *coarctata* and the leaf tubercles of *H. reinwardtii* var. *reinwardtii*. The plant was hexaploid. Since they considered *H. reinwardtii* var. *reinwardtii* a tetraploid, based on Pinto-Lopes's (1946) statement, they felt perhaps the new plant was not a hybrid. They explained: "In order to facilitate the designation of this strain, we suggest the name *Haw. coarctadoidea,* even before the solution of the problem." They referred to it in the legends of their figures as *Haw. coarctatoidea,* but it is listed in Jacobsen (1954) as *H. coarctatoides* and in Jacobsen (1970) as *coartatoides*. Resende and Viveiros (1948) also found some triploid and pentaploid plants that appeared to be hybrids; the former were highly sterile but the latter largely fertile.

6.2 Camera lucida drawing of chromosomes of the triploid *Haworthia resendeana*.

Another species in the Coarctatae section is *H. greenii*. *H. greenii* f. *bakeri* (= f. *greenii*) is larger than forma *minor*, but both are 4*x*, and no difference was found between them in "chromatic mass." Resende and Pinto-Lopes (1946) felt that either the quantitative differences that appear must have been the result of gene mutations, or else the two tetraploid forms came from two diploid plants differing in some genes that, when duplicated in the tetraploids, give two types that are quantitatively different.

A long list of chromosome numbers in *Aloe* was compiled by Müller (1945) from the publications of others. Only *A. ciliaris* had numbers other than $n = 7$ or $2n = 14$. He also included a list of 50 species of *Aloe* and *Leptaloe* and three hybrids he had studied personally, and again only *A. ciliaris* was found to be a polyploid. Müller's species identifications are as authentic as any can be. The plants were largely from the gardens of Dr. G. W. Reynolds of Johannesburg, from the National Herbarium in Pretoria, or from the botany department of the University of Pretoria; all were collected from known regions, mostly from the type localities. Identifications of Reynolds cannot be questioned.

Triploids are uncommon in the Aloineae except in *Haworthia*. The first triploids to be reported were three plants of *Astroloba bicarinata* (Haw.) Uitew. Resende (1937a), who found them, considered them the first and probably was correct. Soon thereafter Resende (1938) reported a triploid plant of *H. resendeana* v. Poelln. (Fig. 6.2), and subsequently several other triploid plants of this species were found. Three varieties of *H. reinwardtii* were found to be triploid or to have triploid forms — notably var. *archibaldiae* (Pinto-Lopes 1944), var. *chalumnensis* (Snoad 1951a), and var. *peddiensis* (Snoad 1951a). Several varieties of *H. limifolia* contain triploid forms — var. *schuldteana* (Riley and Majumdar 1966b), var. *limifolia* (Majumdar 1968), var. *stolonifera* f. *pimentellii* (Majumdar 1968), and a new and unnamed variety studied by Majumdar and Riley (1967). Majumdar (1968) found a triploid plant of *H. nigra* var. *elongata* (Uitew.), and Brandham (1969a) reported one of *H. sampaiana* Res. Triploid hybrids involving *H. limifolia* as one parent were reported by Resende and Viveiros (1948); Majumdar and Riley (1967) found a triploid of a plant identified as *H. bicarneata*, a species not listed in Jacobsen (1954 and 1970). Snoad (1951b) found two unidentified triploids.

The first and only triploid known in *Gasteria* is a hybrid believed to be *G. sulcata* x *G. nigricans* (Riley 1948a). The plant was received from the Huntington Botanical Gardens, and its hybrid nature and putative parents were indicated. However, later studies of the karyotypes of this plant as well as those of *G. sulcata* and *G. nigricans* threw some doubt on its probable origin (Riley, Hammack, and Majumdar 1968). Two identical genomes of the hybrid have karyotypes that look much like that of *G. sulcata* and actually not much different from that of *G. nigricans;* the third genome does not resemble either putative parental species in karyotype. The plant is clearly a triploid, whatever its parents, and has many trivalent configurations; there must be some homology between the

genomes of the two species because two quadrivalents were found in 125 metaphase cells.

An interesting triploid is *Aloe jucunda* from Somalia, as reported by Brandham (1971). He studied meiotic division and found that 83% of the configurations were trivalents; he concluded that the plant was an autotriploid and that nonreduction during the formation of gametes in one parent was a possible cause of its origin.

Another compilation of chromosome numbers revealing polyploidy was made by Snoad (1951a), who studied 236 plants of the Aloineae and combined his data with those previously published by other investigators. In *Aloe* there were four hexaploids and one pentaploid in 87 plants; in *Gasteria* there were three tetraploids and one triploid in 27 plants; but in *Haworthia* there were many more. Of 122 plants in that genus there were five triploids, 24 tetraploids, five pentaploids, 10 hexaploids, one heptaploid, and two octoploids. These results confirm those of earlier reports. Karyotypes of a tetraploid and a pentaploid are illustrated in Figures 6.3 and 6.4.

Resende and Amaral (1956) reported three polyploids; they are a tetraploid, *Aloe* X*lisbonensis;* a pentaploid, *A. gigas;* and a hexaploid, *A. ciliaris*. The first, however, is a known hybrid between *A. ciliaris* and a diploid, *A. gracilis*. The second had previously been published by Resende (1938) under the name *A. ciliaris* f. *gigas*. Most if not all the plants studied up to that time were growing in botanical gardens in various parts of the world, and usually their exact natural locality was unknown. Riley (1958, 1959a) studied 40 plants of *Aloe davyana* from one locality and found only diploids. This was probably the first chromosome study of any wild population of the Aloineae. The plants were growing on the slope of a hill on a farm in the Transvaal at De Wildt, about 48 km north of Pretoria. Six plants were dug up and their root tips were studied; seeds were collected from the other 34 plants and were germinated in petri dishes. The population that was studied occupied an area about 10 m X 10 m in size and was only a small sample of the *A. davyana* growing in that part of the country. Other populations were also observed in the field, but only one or two plants of each species were collected. They included *A. ciliaris,* a hexaploid from the De Bega Valley, Cape Province; *A. ferox* (two plants) from the Kamtra River; *A. mitriformis* from the old Franschhoek Pass road; *A. plicatilis* from the same place; and *A. speciosa* from the region of the Great Fish River.

Two natural populations of *Gasteria* were studied (Riley 1959b), but one was small. Eight plants of *G. beckeri* were collected at the Kamtra River in the Alexandria District of the Cape Province, not far from Grahamstown; all were diploids. At the Bushmen's River Poort, not far from the other locality, 50 plants were collected; they were undoubtedly all of the same species or variety and were close to *G. zeyheri,* but a more exact designation was not made although a botanist from Rhodes University (who knew the plants of the area) was present. The closer botanists are to plants in the field, the more hesitation they seem to have in identifying them. Seven plants of *Haworthia reinwardtii* also were collected at the Kamtra River. They were probably one of the many varieties other than var.

6.3 Camera lucida drawing of chromosomes of the tetraploid *Haworthia recurva*.

6.4 Camera lucida drawing of chromosomes of the pentaploid *Haworthia revendetii*.

reinwardtii, but the South African botanist refused to try to identify them more closely; she felt identification of named varieties of this species was a task only for *Haworthia* specialists. All the *Gasteria* and *Haworthia* plants collected in the field were diploids.

In 1959 the senior author (Riley 1959c) compiled a list of chromosome numbers in *Aloe*. Of 91 South African species and varieties listed in Reynolds (1950) and/or Jacobsen (1954), only two polyploid species were reported. One was *A. ciliaris*, which is also listed as an aneuploid; the other was *A. tidmarshii* (Schoenl.) Müller, which seems to occur in both diploid (Müller 1945) and hexaploid forms (Snoad 1951a). This latter species, although often cited as *A. tidmarshii* (Schoenl.) Müller, is often considered a mutant form of *A. ciliaris;* so perhaps there is only one polyploid species in the list. One horticultural form and 19 putative hybrids showed no polyploidy. There was one polyploid (*A. gigas* = *A. ciliaris* f. mut. *gigas* Res.) in 19 plants from countries other than South Africa and one (*A. tenuifolia*) among 11 species not listed in Reynolds or Jacobsen. In 36 unidentified plants Snoad (1951b) recorded one pentaploid and four hexaploids, but these findings have no meaning since the plants were unidentified; the hexaploids might all be *A. ciliaris*.

The following year a report was made on *Haworthia* (Riley 1960), and the same complicated situation was found as had been reported earlier. Of 110 species and varieties there were three triploids, 18 tetraploids, three pentaploids, four hexaploids, three octoploids, and one nonaploid. Three species had 2*x* and 4*x* forms; three had 2*x* and 6*x* plants; one had 2*x*, 4*x*, and 6*x*; and one species was 2*x*, 4*x*, 5*x*, 6*x*, and 8*x*. Nearly all the polyploids were in the Coarctatae and Tessellatae sections, with an occasional one in the Limifoliae and Margaritiferae sections. Three horticultural varieties were diploids. Of nine putative hybrids two were 6*x* and one was 7*x*, and in one hybrid there were 3*x* and 5*x* forms. All the polyploid hybrids involved the Coarctatae or Tessellatae sections in one or both parents. There were one triploid, two tetraploids, one pentaploid, three hexaploids, one heptaploid, and one octoploid in 24 species not in Jacobsen (1954). In *Gasteria* (Riley 1961) of 44 species and four varieties, two species had both diploid and tetraploid forms and one variety was a tetraploid. One horticultural variety and 11 plants not listed in Jacobsen (1954) were all diploids. Of 11 interspecific hybrids one was a triploid and one had both diploid and tetraploid forms, and there were one triploid and four tetraploids of 132 unidentified plants. In *Astroloba* four species were 2*x*, one was 4*x*, and two were aneuploid; two unidentified plants were 2*x*. One plant of *Poellnitzia* was 2*x*.

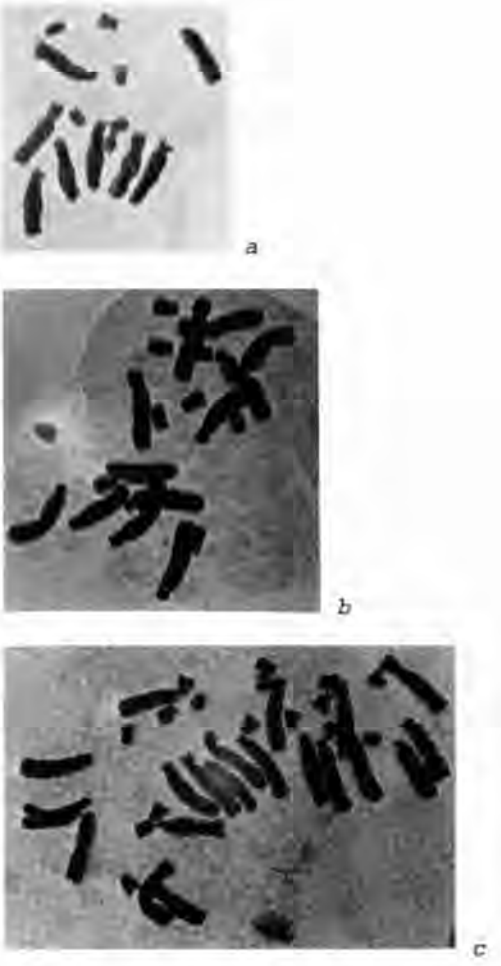
Several intergeneric hybrids were available for study (Riley 1961). Three *Gasterhaworthia* hybrids were diploids. Nine putative hybrids between *Gasteria* and *Aloe* were diploids, and one (apparently synthesized in Japan) had diploid and tetraploid forms. A plant apparently *Gasteria* X *Astroloba* had "about 14 chromosomes." These tabulations were based on reports by other investigators and therefore include plants previously discussed in this chapter.

Sharma and Mallick (1965) more recently recorded chromosome numbers of 32 *Aloe*, three *Haworthia*, four *Gasteria*, and one *Gastrolea* species, most of which had been counted previously. Only *Aloe ciliaris* ($2n = 42$) and *A. humilis* ($2n = 21$) were polyploids. Riley and Majumdar (1965a) reported several polyploids, most previously recorded; *Haworthia tessellata* var. *parva* subv. *major* was a tetraploid not studied previously, and *H. mirabilis* was a hexaploid and the first polyploid reported for the Retusae section. Fifteen new determinations were made by Riley and Mukerjee (1965) for *Haworthia;* four were polyploids, all in the Coarctatae section. A few other polyploids were added by Majumdar and Riley (1967). The chromosomes of diploid, triploid, and pentaploid species of *Haworthia* are shown in Figure 6.5.

The results of studies of the main summaries made of *Aloe, Astroloba, Gasteria,* and *Haworthia* before 1970 are listed in Table 6.1. They illustrate what we have previously stated, that there is considerable variability among these four main genera. *Aloe* has the smallest number of polyploids, *Gasteria* is next with slightly more, and the amount of polyploidy is high in the other two genera. However, *Astroloba* has so few species that it is not particularly significant, and in *Haworthia* the large amount of polyploidy is largely concentrated in four sections. It is interesting that in all four genera the percentage does not vary greatly from one author to another, although no statistical tests for significance were thought to be worth making. Later studies tend to resemble the earlier ones to some extent since most of them include earlier results; all except Riley's (1959c, 1960, 1961) summaries also contain new material.

A recent extensive study of chromosome numbers in the Aloineae has been published by Brandham (1971), who covered 228 species, varieties, and hybrids and 16 unidentified plants. These were determinations made by him, and many of the taxa had not been studied previously except for the present junior author's doctoral dissertation (Majumdar 1968). The results follow the general pattern of low polyploidy in *Aloe* and *Gasteria* and a much higher percentage in *Astroloba* and *Haworthia*. Brandham found nearly twice as many polyploids in *Aloe* as had been reported by other investigators and about the same percentage as previously found in *Gasteria*. In *Astroloba* he found a smaller percentage of polyploids, but his numbers were small; he found a smaller percentage in *Haworthia* also, although not very much smaller. When he recorded the Coarctatae and Tessellatae sections separately he found they contained 66% polyploids, whereas all the other sections taken together had 6.3% — about the same as in *Aloe* and *Gasteria*.

Some previous misconceptions of the nature of polyploidy in the Aloineae group show that it is generally unwise to make broad generalizations on earlier studies based on a small amount of material. Kostoff (1939) stated that species with gigantic chromosomes do not have large numbers of chromosomes. He cited *Haworthia* as an example and mentioned that all species of the genus studied up to that time were diploids and therefore did not have large chromosome numbers. That genus was an unfortu-



6.5 Photomicrographs of chromosomes of: (*a*) the diploid *Haworthia fasciata;* (*b*) the triploid *H. limifolia;* (*c*) the pentaploid *H. tessellata*.

TABLE 6.1 Numbers of Diploid and Polyploid Species and Varieties of *Aloe*, *Gasteria*, *Haworthia*, and *Astroloba* Published in Major Summaries before 1970. (Hybrids and Aneuploids Not Included.)

	Species and Varieties											
Genus	2*x*	3*x*	4*x*	5*x*	6*x*	7*x*	8*x*	9*x*	2*x* and Polypl.*	Mixed Polypl.†	Total	Polyploids (%)
Aloe												
Ferguson (1926)	7										7	0.0
Sato (1937)	15										15	0.0
Resende (1937a)	83				1						84	1.2
Resende (1940a)	87			1	1						89	2.2
Müller (1945)	127				1					1	129	1.6
Snoad (1951a)	83			1	4						88	5.7
Riley (1959c)	89				1				1		91	2.2
Total											503	2.4
Gasteria												
Ferguson (1926)	11		1								12	8.3
Sato (1937)	15		1						1		17	11.8
Resende (1937a)	20		1								21	4.8
Resende (1940a)	34		1						3		38	10.5
Snoad (1951a)	23	1	3								27	14.3
Riley (1961)	45		1						2		48	6.2
Total											161	8.1
Haworthia												
Ferguson (1926)	10		5								15	33.3
Sato (1937)	17		2						1		20	15.0
Resende (1937a)	31		2		1				2		36	13.9
Resende (1940a)	42	1	10		3				2	1	59	28.8
Snoad (1951a)	75	5	24	5	10	1	2				122	38.5
Riley (1960)	67	3	18	3	4		3	1	8		107	37.4
Majumdar (1968)	97	3	23	3	2						128	24.2
Total											485	29.7
Astroloba												
Ferguson (1926)	2		1								3	33.3
Resende (1937a)	4	1	1								6	33.3
Resende (1940a)	6		2								8	25.0
Riley (1961)	4	1	2								7	42.9
Total											24	33.3

*This column includes species with 2*x*, 4*x*, 2*x* + 6*x*, 2*x* + 4*x* + 6*x*, or 2*x* + 4*x* + 5*x* + 6*x* + 8*x* forms.

†One species has 4*x* and 6*x* and one has 5*x* and 6*x* forms.

nate choice since it contains sections with the highest numbers in the Aloineae. As a whole Kostoff's generalization is unsound; there are too many examples of this group with more than 4*x* chromosomes (4*x* = 28), even though the chromosomes are very large. Darlington (1956) also has indulged in some generalizations, stating that changes in chromosome number are less frequent in long-lived than in short-lived plants. Apparently this statement is true of trees and shrubs, but the Aloineae are long-lived plants and numerous changes in chromosome numbers have occurred in them.

The origin of natural polyploids cannot usually be determined. Resende and Viveiros (1948) studied *Haworthia*

TABLE 6.2 Numbers of Diploid and Polyploid Plants of the Aloineae Reported by Brandham (1971)

	Species and Varieties*								
Genus	2x	3x	4x	5x	6x	2x and 3x	2x and 4x	Total	Polyploids (%)
Aloe	101	0	4	0	1	1	0	107	5.6
Astroloba	4	1	0	0	0	0	0	5	20.0
Chamaealoe	1	0	0	0	0	0	0	1	0.0
Gasterhaworthia	1	0	0	0	0	0	0	1	0.0
Gasteria	33	0	2	0	0	0	1	36	8.3
Gasteria hybrids	1	0	0	0	0	0	0	1	0.0
Gastrolea	2†	0	0	0	0	0	0	2	0.0
Haworthia	53	3	10	2	4	0	0	72	26.3
Haworthia hybrids	1	0	0	0	0	0	0	1	0.0
Lomatophyllum	1	0	0	0	0	0	0	1	0.0

Unidentified plants:
Aloe: 2x = 5 plants; 4x = 1 plant
Astroloba: 2x = 1 plant
Gasteria: 2x = 9 plants

*Several species were studied from more than one locality, but each species is listed only once.

†*Gastrolea novotnyi* has 2n = 20 chromosomes; it is not included in this table.

limifolia var. *schuldteana* and *H. limifolia* var. *limifolia* (= *H. limifolia* var. *marlotheana*) and described characters they assumed to represent quantitative differences. Because of these presumably quantitative differences they assumed that these two varieties *"show us a typical example of autopolyploidy found spontaneously"* (their italics). Whether their reasoning is correct is highly questionable, as is their statement that the characters are quantitative.

Brandham (1971) has also suggested a possible mode of origin of the large number of polyploids in *Haworthia*. He points out that the frequency is much greater in the Coarctatae and Tessellatae sections, "probably due to selection of polyploids during a long period of cultivation." He adds: "These sections are particularly prized as vegetative ornamentals, and many subspecies and varieties are artificial and are known only in cultivation. New vegetative variants of these ornamentals brought about by polyploid formation (among other factors) would be given preference and would raise the incidence of polyploidy to a level higher than that found among wild plants."

It is true that if more desirable mutations arose in collections of succulent fanciers they would tend to be propagated for their commercial value. If these mutants were polyploid varieties, the percentage of polyploid plants in that collection would be increased greatly. However, it has been the experience of the present writers that the polyploid species or varieties of *Haworthia* are no more beautiful and no more interesting than the diploid forms of the same or of closely related species and varieties and that some species (*H. armstrongii, H. coarctata, H. jonesiae, H. reinwardtii,* and *H. tessellatae*) comprise several polyploid as well as diploid types which

are indistinguishable morphologically. More recently Brandham (personal communication) has seen many hundreds of wild-collected haworthias which show a pattern of polyploidy similar to that occurring among cultivated plants.

The possibility that a disproportionate number of polyploids might be found in a commercial collection because of artificial selection by commercial growers of succulent plants is a compelling reason for not trying to base phylogenetic studies on that type of collection. Collections in botanical gardens should be better in that they are often taxonomically oriented and usually try to be complete for all available species to the exclusion of others. The plants the authors received from J. W. Dodson of California were not primarily from a commercial source, although the International Succulent Institute does sell plants. Dodson himself is more than a succulent fancier; he is a well-known taxonomist of the Aloineae, as indicated by the species *Astroloba dodsoniana* Uitew. named after him. The senior author's original work in the Aloineae was with *Gasteria,* but his year in South Africa in 1955-56 and his more recent observations in the Great Fish River Valley broadened his interest to include other genera, especially *Haworthia*. When he decided to include this group, he inquired about the possibility of obtaining haworthias from Hans Herre and other South African botanists. They pointed out that haworthias must be shipped by air (although, as the senior author found out, gasterias may readily go by sea) and that air express is costly. Herre kindly pointed out that he had shipped and was continuing to ship many haworthias to Dodson. The latter Aloinologist kindly placed hundreds of his plants at the writer's disposal for scientific purposes. These plants had not been lying around in a succulent collection for many years, and they had not been propagated for their beauty. Even if polyploids were more desirable than diploids, which the writer doubts, Brandham's criticism would not apply to this collection.

Chapter Seven

ANEUPLOIDY

Polyploids have somatic or sporophytic chromosome numbers that are multiples of the haploid number other than the diploid number. If the number in the haploid set is x (i.e., if $n = x$), polyploid plants might have $3x$, $4x$, $5x$, or some other multiple (i.e., $2n = 3x$, $4x$, $5x$, and so forth). Aneuploids resemble polyploids in that they also have extra or fewer whole chromosomes, but they differ in that their number is not an exact multiple. Thus their sporophytic numbers might be $2n + 1$ (trisomic), $2n + 2$ (tetrasomic), $2n + 1 + 1$ (double trisomic), $2n - 1$ (monosomic), $2n - 2$ (nullosomic), and so forth.

Chromosome configurations at meiosis vary in aneuploids according to the presence or absence of chromosomes and the number of extra ones. Thus in a trisomic plant all the chromosomes would be arranged in pairs on the first metaphase plate except the one that was trisomic, and this chromosome would usually be in a trivalent configuration with all three chromosomes arranged in a group. Similarly a double trisomic would usually have two trivalent configurations, and a tetrasomic would frequently have a quadrivalent configuration with four chromosomes arranged in a ring or chain of four. A monosomic plant would have one chromosome missing, and its homologue would be alone on the metaphase plate as a univalent.

The first member of the Aloineae known to have a chromosome number other than the haploid, diploid, or polyploid number was *Aloe ciliaris,* which was cited by Ferguson (1926) as having >45 chromosomes "in somatic (diploid) nuclei." This number is listed in her Table I, but there is no reference to it in the text. The plant appears to be a hyperhexaploid.

A different number was given for this species a few years later by Gioelli (1930), who found n = >25. Tischler (1931) cited Gioelli (1930) in his list of chromosome numbers as reporting >25 for the haploid chromosome number of *Aloe ciliaris;* Resende (1940a) in his list of $2n$ numbers gave >50 for this species, also citing Gioelli, and >45 from Ferguson. Although the plants of both Ferguson (1926) and Gioelli (1930) were apparently aneuploids, most of the plants of this species are hexaploids. A $2n$ ($6x$) number of 42 has been given by a number of authors, as listed in Chapter 5.

In *Haworthia sampaiana* Res., Resende (1938) reported 39-40 chromosomes. This plant is clearly an aneuploid.

In *H. glauca* Baker, he listed $2n = 29$, indicating that it is a hypertetraploid.

A clue to the occasional origin of aneuploids was given by Marshak (1934), who found some pollen grains of *Haworthia planifolia* and *H. brevifolia* with an additional chromosome. In *Gasteria sulcata* a few pollen grains had $n - 1$ chromosomes, lacking one of the short ones, and in *Astroloba congesta* the usual count seemed to be six long and three short chromosomes. Matsuura and Suto (cited in Suto 1936) found that some pollen grains in a plant of an unidentified species of *Aloe* had one long chromosome in addition to the normal chromosome constitution. Suto also cited Marshak's references to aberrant pollen grains. Suto mentioned that *Gasteria brevifolia* and *G. planifolia* have occasional pollen grains with additional chromosomes and referred to Marshak's paper as his authority. However, Marshak listed *Haworthia brevifolia* and *H. planifolia* and not those species of *Gasteria* as having pollen grains with additional chromosomes. In Jacobsen (1970) no *H. brevifolia* is listed. Therefore, Marshak's reference to it must be an error and Suto was probably correcting the error. There is a species of *planifolia* in each of those genera, however, so it is difficult to tell whether Marshak made an error with this species or whether Suto misquoted a correct reference. *G. planifolia* Bak. was once placed under *Aloe* as *A. planifolia* Bak., and *Haworthia planifolia* Haw. was once classified as *Aloe planifolia* Roem. & Schult. The taxonomic situation cannot now be corrected since Marshak studied both *G. planifolia* and *H. planifolia,* but this whole situation illustrates the futility of trying to base a biosystematic study on greenhouse material.

Kondo and Megata (1943) have published a fairly extensive list of chromosome numbers in the Aloineae. Under *Gasteria nigricans* Haw. they include one reference showing three quadrivalents, five bivalents, and two univalents in the first meiotic division; these configurations add up to 24 chromosomes, which makes this plant clearly an aneuploid. Unfortunately their reference is in Japanese and the present writers have been unable to read the text.

Müller (1945) extended his earlier study and included an elaborate table which lists the results of previous investigators; it includes 76 species and varieties and eight known or putative hybrids. In this list the only aneuploid mentioned is Ferguson's (1926) number of 45 chromosomes in *A. ciliaris* Haw. A second table included only Müller's own studies. In 50 species and varieties and three known hybrids he reported no aneuploids and only one polyploid (*A. ciliaris,* $2n = 42$). Sixteen of the species he studied had been studied previously.

Pinto-Lopes (1946) listed the chromosome numbers of 32 species and varieties of *Haworthia,* including nine varieties of *H. reinwardtii*. The only aneuploid was *H. sampaiana* Res., which had $2n = 36$, or $5x + 1$. Resende (1938) had reported the sporophytic number of this species as 39-40 and had cited it as the first aneuploid species found in the Aloineae, apparently ignoring Ferguson's puzzling report for *Aloe ciliaris*. Pinto-Lopes reported he was able to count 21 large and 15 small chromosomes "without giving rise to any doubt whatsoever." In his Table-summa-

ry No. 1 he noted that he had "corrected" Resende's number, although it is possible that he and Resende were dealing with different clones. In general the number of chromosomes in this species appears to be unstable: Brandham (1969a) found 21 as the sporophytic number; Majumdar (1968) found 35; and Pinto-Lopes (1944, 1946), Resende and Pinto-Lopes (1946), Snoad (1951a), and Viveiros (1959) found 36. This lack of uniformity suggests that Resende's number of 39 or 40 could possibly also be correct for his clone and did not necessarily need to be corrected by Pinto-Lopes. Pinto-Lopes (1946) found that the $2n$ chromosome number of his plants of *H. glauca* Bak. was 28. Inasmuch as Resende (1938) found 29 chromosomes in the sporophytic cells of that species, two numbers have been found in different specimens of the species — tetraploid and hypertetraploid. A more recent study by Riley and Majumdar (1968) showed a plant of this species to be a tetraploid with two reciprocal translocations of the centric fusion type.

Pinto-Lopes (1946) discussed *H. sampaiana* f. *broteriana* (as *H. broteriana* Res.), stated that it had 25 chromosomes, and then added, "The species is, therefore, pentaploid." In his Table III and Table-summary No. 1 the $2n$ number of this species is given as 35. Therefore, the number 25 given on page 191 is clearly a misprint, and the plant should not be cited as an example of an aneuploid.

The origin of some of these polyploid forms has been confusing. Resende and Pinto-Lopes (1946) found that although *H. sampaiana* f. *broteriana* was a pentaploid ($2n$ = 35) and *H. sampaiana* was a hyperpentaploid ($2n$ = 36), both produced fertile seeds (in Lisbon, Portugal) and *H. sampaiana* apparently gave a number of forms of different numbers of chromosomes including some pentaploids which differ from one another morphologically. Among them may be *H. revendettii* Uitew., but it differed so much from *H. sampaiana* and f. *broteriana* "in the appearance of the leaves as well as in the arrangement of these" that Pinto-Lopes stated, "For the present we shall not consider this strain as belonging to the same species as the other two." He suggested that "these pentaploids" probably came from a $4x \times 6x$ cross but that the tetraploid and hexaploid species known in the Coarctatae section up to that time were so different from them phenotypically that "both, or, at least, one of the parents must still be unknown to Systematics."

Viveiros (1949) tabulated chromosome numbers published for *Haworthia* before 1949 and listed four aneuploids. One was *H. sampaiana,* and Viveiros listed both Pinto-Lopes's (1944) number of $2n$ = 36 and Resende's (1938) number of $2n$ = 39-40, not recognizing Pinto-Lopes's statement that he had "corrected" Resende's report. He also listed three new varietal numbers he had just found: 58(?) for *H. tessellata* var. *coriacea* f. *brevior,* 61(?) for *H. tessellata* var. *coriacea* f. *longior,* and 63 for *H. tessellata* var. *luisieri*. Viveiros (1959) also found some aneuploid plants in the F_1 seedlings from several species, but since they did not occur in nature, they will not be discussed here.

Brandham and Johnson (1977) found nine plants of *Aloe elgonica* with 29 chromosomes; three had an additional long

chromosome and three had an additional short one. Three others had an additional short chromosome and were therefore aneuploid, but also contained a short-arm deletion. The deletion will be discussed in greater detail in the next chapter and the whole population in which they occurred will be described in Chapter 12.

Meiotic studies in *Gasteria* combined with those of the first postmeiotic microspore division (Riley 1959d) gave some indication of the possible origin of aneuploid plants; 24 species or varieties and ten interspecific hybrids all had 14 somatic chromosomes. Meiotic abnormalities at the first meiotic metaphase were more numerous in two hybrids and one species than in the other plants, but in most of the plants aberrations were few. Pairing was generally regular and failure of pairing was uncommon except in two of the hybrids. In a few cells there were only six long chromosomes, and in one hybrid one cell had ten; more frequent was one pair of short chromosomes too many or one pair too few. These aneuploid aberrations apparently arose at a mitosis just before the meiotic divisions and could result in microspores with fewer than the normal number of chromosomes and therefore in aneuploid plants.

In 1962 Riley and Mukerjee published a study of two newly discovered aneuploids in *Haworthia* — a hypotetraploid and a hypohexaploid. *H. reinwardtii* var. *chalwinii* (Marl. & Bgr.) Res. had 14 long chromosomes and 12 short ones and was therefore $4x - 2$ in number. Other investigators (Resende 1937a, 1938; Pinto-Lopes 1946; Snoad 1951b) had found that the number $2n$ was 28 in other plants of the same species. Later Riley and Mukerjee (1965) found a plant of that species with 28 somatic chromosomes. In a plant of *H. tessellata* var. *engleri* there were 40 somatic chromosomes, which represented the condition $6x - 2$. There were 22 long chromosomes and 18 short ones; Resende (1940a) had previously found in another plant of the same species that $2n = 28$, and later Riley and Majumdar (1966a) found a plant with the same chromosome number as Resende's. It is interesting that in both plants it is long chromosomes and not short ones that are missing, which seems to fit the general pattern. Only a few other aneuploid plants have been found, but it is usually the long chromosomes that are missing or in excess. Perhaps the reason is that in a polyploid the long chromosomes, because they are long, get tangled and do not separate properly. Of course all these aneuploids are hypo- or hyperpolyploids, because hypo- or hyperdiploids probably could not survive.

Up to 1965 several aneuploids had been reported in the literature, including *Aloe ciliaris* (Ferguson 1926), *Astroloba pentagona* var. *spiralis* (Ferguson 1926), *As. congesta* (Marshak 1934), *Gasteria nigricans* (Kondo and Megata 1943), *Haworthia glauca* (Resende 1938), *H. sampaiana* (Resende 1938; Pinto-Lopes 1944, 1946; Snoad 1951a), *H. tessellata* var. *coriacea* f. *brevior* and f. *longior* (Viveiros 1949), *H. tessellata* var. *luisieri* (Viveiros 1949), *H. tessellata* var. *engleri* and *H. reinwardtii* var. *chalwinii* (Riley and Mukerjee 1962). The first nine of these were included in lists of chromosome numbers published by the senior author up to 1961 (Riley 1959c, 1960, 1961), and

the last two were published in the 1962 paper (with Mukerjee) in the *Journal of Heredity*. In spite of this list of eleven aneuploids, Sharma and Mallick (1965) wrote, "Riley's analysis shows that the genus *Gasteria* is characterised by almost universally diploid species, *Aloe* by mostly diploid and a few polyploid, and *Haworthia* by diploid with a considerable frequency of polyploids. In spite of the presence of a large number of species...not a single case of aneuploidy has been reported. The striking uniformity in cytology makes the Aloineae a natural assemblage." It is difficult to understand how Sharma and Mallick ignored these eleven examples, since aneuploidy was certainly not unknown in 1965.

Another aneuploid was reported in 1965 (Riley and Mukerjee 1965). It was in a clone of *Haworthia greenii* f. *pseudocoarctata*. Pinto-Lopes (1946) had studied this form and found $2n = 28$. In Riley and Mukerjee's study two different clones were available which happened to be numbered 6 and 12; clone 12 was a perfectly normal tetraploid, like Pinto-Lopes's, but clone 6 had 30 chromosomes in root-tip cells and there were 14 short chromosomes instead of 12. This plant differed from some of the earlier aneuploids studied with respect to the number of short rather than long chromosomes.

A variety of *Haworthia limifolia*, with 23 somatic chromosomes in all the cells of the root tip, is the twelfth aneuploid in the Aloineae the writers have encountered (Riley and Majumdar 1966b; Majumdar and Riley 1967; Majumdar 1968) either directly or in the literature. It was received from the International Succulent Institute and was regarded as a new and unnamed variety of *H. limifolia*. Measurements of all the chromosomes in the cell showed the L_2 and L_4 chromosomes were present four times whereas there were only three of each of the other chromosomes, so it was $3x + 1 + 1$. There were trivalents, as one would expect in a triploid plant, and quadrivalents, which probably resulted from the aneuploid situation; there was also a puzzling occasional hexavalent. In general, meiosis was quite irregular. Chromosome numbers in the microspores showed considerable variation, and this could be the source of the origin of further aneuploids. Generally, though, the irregular behavior of the chromosomes of this plant resulted more from its triploid than its aneuploid condition. Pollen fertility was about 18%, and about 60% of the healthy-looking pollen germinated in artificial culture. No seeds were obtained from self-pollination, but this might be explained by the fact that the Aloineae are all or almost all self-incompatible.

In a personal communication Brandham has included some of his more recent unpublished results. He has now looked at over 1,500 diploids of wild origin and has found only a single plant (*H. attenuata*) with an additional short chromosome ($2n = 15$). In tetraploid aloes from East Africa he has found that aneuploids are moderately common (about 5% of the individuals), with $2n = 27$ or 29 being found. The loss or gain of short chromosomes is more frequent, but the loss or gain of long chromosomes also occurs. In *Haworthia* he has found no aneuploid polyploids except $2n = 40$ and 43 in *H. glauca*. He reports that aneuploidy is common in artificial hybrids involving polyploid

aloes. Diploid x triploid has given $2n$ = 14, 15, 16, and 19 in progeny surviving to maturity. Similarly he found that triploid x tetraploid has given $2n$ = 25, 27, 28, and 36(!), the last being an unreduced triploid gamete fertilized by a 15-chromosome gamete from the tetraploid. Tetraploid x tetraploid gave $2n$ = 27, 28, 29, 30; tetraploid x diploid gave $2n$ = 20, 21, 22, 23. In these last two crosses the triploid was Brandham's slightly fertile autotriploid *A. jucunda*.

As might be expected from the situation with respect to polyploids, most of the aneuploids of *Haworthia* were found in the Coarctatae and Tessellatae sections. Of the nine *Haworthia* aneuploids, four were from the Coarctatae section, one from the Limifolia section, and four from the Tessellatae section.

An intergeneric aneuploid hybrid was recently reported by Brandham (1969a, 1971): x*Gastrolea novotnyi* Walth. has 20 somatic chromosomes and is a hypotriploid.

In one plant of *Aloe barbadensis* Mill. (= *A. perfoliata* var. *vera* L. = *A. vera* "L.") Vig (1968) found two pairs of the short chromosomes missing in some sections of the roots; the roots appeared to be sectorial chimaeras, having 14 chromosomes in some regions and ten in others, with both numbers intermingled in still others. In sections with the two pairs missing, the two short chromosomes present were different from one another; one was a rather typical short chromosome and had a median centromere, and the other was almost intermediate in length and had a submedian centromere. Brandham (personal communication), on the other hand, examined several accessions of this species and found that all had normal karyotypes.

Chapter Eight

DELETIONS, DUPLICATIONS, AND INVERSIONS

Polyploids and aneuploids are aberrations that involve whole chromosomes. However, some chromosome aberrations involve only pieces of chromosomes; they belong to types known as deletions or deficiencies, duplications, inversions, and translocations.

Deletions

Deletions or deficiencies are aberrations in which one or more segments of a chromosome or chromosomes are missing. According to Rieger, Michaelis, and Green (1968), the term deficiency should be used if the missing piece is a terminal acentric segment of a chromosome, chromatid, or subchromatid, whereas deletion is properly applied only if the missing segment is intercalary. But this nice distinction is often ignored. Swanson (1957), for example, stated that "deficiencies can be either terminal or interstitial." De Robertis, Nowinski, and Saez (1970) defined a deficiency as an aberration that has a missing piece, "either *interstitial* or *terminal*," and immediately referred to it as "the deleted segment." Brown and Bertke (1969) similarly wrote, "Deletions may be terminal or interstitial."

Deletions may occur spontaneously or may be induced by subjecting a plant or animal to ionizing radiation or to certain chemical compounds. A terminal deletion would result from a single break in a chromosome or chromatid and thus produce an acentric fragment and a chromosome that is normal except that it lacks this fragment. An intercalary or interstitial deletion results from two breaks. The segment between the breaks drops out, the two broken ends of the remainder of the chromosome join to form a chromosome or chromatid that appears to be normal except that it has a shortened arm, and the two broken ends of the missing intercalary segment also fuse so that a ring-shaped chromosome is produced. If one break is in one arm and the other break is in the other arm, the piece between the breaks will round up into a small centric ring while the

two ends will fuse into an acentric rod-shaped fragment.

In mitosis, deletions can be identified only if they are of some size, since the deleted chromosome behaves like an unbroken chromosome. As the chromosomes shorten and thicken, the deleted and normal chromosomes may look alike on the metaphase plate; if the deleted piece is large, the difference is readily detected. If the deleted piece is a ring without a centromere, it may be noticed in the cytoplasm near the spindle. In meiosis the deletion, unless it is very small, is easily detected at pachytene in those organisms in which the pachytene stage is clear. Since the rule of synapsis is part-by-part pairing, the terminal deletion is clear since one of the two homologues will appear longer than the other. If the deletion is intercalary, homologous parts will pair throughout, and the part of the normal chromosome that corresponds to the missing part of the deleted chromosome will bulge out into a side loop since it has no homologous segment with which to pair. If the missing segment is fairly small, the corresponding piece may not loop out, and part of the deleted chromosome may stretch over the unbulged undeleted part of the homologue; this arrangement may necessitate some nonhomologous pairing on either side of the deletion. A very small deletion may not be detected, because the deleted and normal chromosomes may be almost the same size and the deleted fragment may be too small to be noticed.

In organisms with polytene chromosomes such as are found in the salivary glands and some other tissues of *Drosophila melanogaster,* deletions can be recognized easily. Those giant chromosomes are marked with crossbands, and it is easy to see if some of the bands are missing. Also loops are present corresponding to those seen in the pachytene stage of meiosis, and the nature of the loops and of pairing is easily observed from a study of the bands.

A deficiency may be homozygous if it is present in both members of a homologous pair, or heterozygous if it is present in only one. Homozygous deficiencies are nearly always lethal, although occasionally a few small ones survive, such as the deletions causing the loss of the yellow, achaete, and scute genes in *Drosophila melanogaster* and one deletion in *Zea mays*. Homozygous deletions in animals generally do not survive to the adult stage, and these deletions in *Drosophila* are exceptions. In plants the problem of homozygous deficiencies in the sporophyte generation usually does not arise because deficiencies often do not survive the gametophyte stage, especially on the male side. Usually pollen grains that bear the deficient chromosome do not develop normally; those that happen to do so usually do not produce pollen tubes that compete successfully with normal ones. In a few plants pollen grains bearing a deficiency may actually produce good pollen tubes that bring about fertilization, and the endosperm or the embryo will carry the deficiency. If a deficient chromosome is transmitted successfully through the egg but not through the pollen, heterozygous deficient plants will arise; if through both the egg and sperm nucleus, some of the offspring may be homozygous deficient; but if neither eggs nor pollen will be viable with the deficient chromosome, only normal plants will result but ap-

proximately 50% of the eggs and pollen will be inviable.

In plants in general, deletions are more likely to survive and be found in tetraploids than in diploids, probably because they do not represent such a great unbalance in the genotype. Brandham (personal communication) had found that small and large pieces missing from the chromosomes of tetraploid aloes are quite common, both in wild plants and in artificial hybrids. They appear to be lethal in diploids, although one very small deletion has been identified, and they also occur in tetraploid haworthias.

Because of the lethal effect, most deletions or deficiencies are not very important in evolution, with perhaps one exception. Many deficiencies, such as the "minutes" in *Drosophila melanogaster,* show a phenotypic effect, in that way resembling gene or "point" mutations. Therefore, in some organisms very small deletions may behave as point mutations and thus may be of some importance in evolution, although, because they are often lethal when homozygous, they probably as a whole contribute little to the problem of the origin of species.

As mentioned many times previously, the normal haploid karyotype of the various species of the Aloineae consists of four long and three short chromosomes. In one species — *Aloe barbadensis* Mill. (= *A. perfoliata* var. *vera* L. and often erroneously called *A. vera* L.) — there may be three long, one intermediate, and three short chromosomes. This configuration was first reported by Marshak (1934). It may have resulted from the loss of a terminal or intercalary chromosomal segment; if so, the fragment that initially accompanies such deficiencies has long been lost. Marshak pointed out that most of the aloes are African, with a few in southern Arabia, but this species is found around the Mediterranean Sea and covers a territory from the Canary Islands to China and Taiwan. There is considerable doubt and some controversy whether *A. barbadensis* is indigenous to that vast region. Marshak suggested that even if it is not, "it is striking to note that it alone should have been able to survive and that there is correlated with this different survival value a difference in its chromosomal constitution." The origin of this karyotype is lost in antiquity, but the fact that one chromosome is considerably shorter than the others suggests (but only suggests) a deficiency that may have a peculiar survival value under strange surroundings. This problem is highly speculative and Marshak's idea may well be a matter of *post hoc, ergo propter hoc.*

In a more recent study of the same species, Vig (1968) reported that the normal haploid chromosome number consists of four long and three short chromosomes. He made no mention of any intermediate-sized chromosomes and showed eight long ones in his figures. In some sections of the roots two of the short chromosomes were missing, and the short chromosomes that were present differed in size and in the position of the centromere. This plant had a normal chromosome complement insofar as the long chromosomes were concerned. It is probable, therefore, that Marshak's plant was aberrant. Brandham (personal communication) has found no aberrant karyotype in this species.

A rather unusual deletion was pointed out by Pinto-Lopes and Resende (1949). They found breaks in the nucleolus constriction (called by them the "olistherozone") which removed the satellite; this situation actually was a translocation in which the segment was transferred from one chromatid to the sister chromatid, thus producing a deleted and a duplicated chromatid. Resende and Manarte (1951) suggested that the distensions of the chromatids produced by agglutination cause fractures in chromatids and half-chromatids. This fracturing apparently results from the pull on a bridge, and this distension produces "kalymmatic rarefaction and despiralizations" which presumably cause the breaking of the chromatid. Their terms are little used.

Majumdar (1965) in his thesis noted a possible deletion in *Haworthia fasciata*. This species had the normal karyotype of eight long and six short chromosomes, but one of the L_1 chromosomes had a shorter long arm than the other; the same was true of the L_3 chromosomes. Perhaps the shorter chromosomes of these heteromorphic pairs represent deletions. This species has also been described by Majumdar and Riley (1967) and Majumdar (1968). In *H.* aff. *baccata* the situation is confusing. There is apparently only one L_1 chromosome, but there seem to be three L_3 chromosomes. Since the chromosomes are compared and designated by their short arms, it is possible that one of the apparent L_3 chromosomes is actually an L_1 chromosome with a small deletion in its short arm. The plant of *H. fasciata* was almost 100% male sterile. Meiosis appeared to be normal, but most of the microspores appeared to be unhealthy. The plant of *H.* aff. *baccata* was little better with only 17% good pollen. In both plants the low percentage of fertility might have been caused by deletions, since they have been known for years to cause sterility. Many other plants have been shown by many authors to be partially pollen sterile, and the cause may be some small, otherwise undetected deletions.

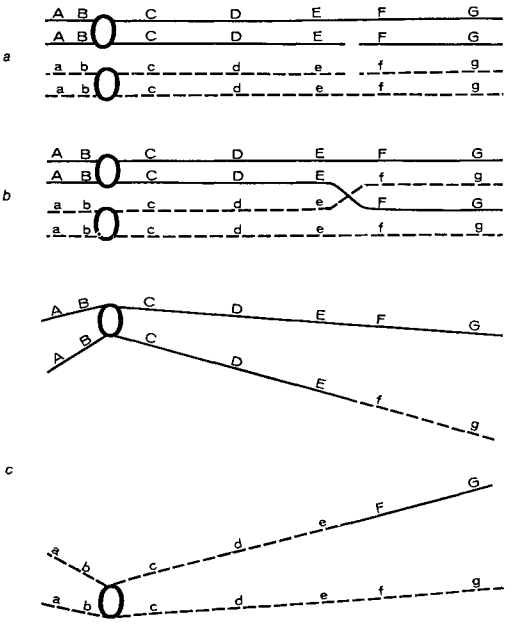
A similar broken arm has been reported in *Haworthia resendeana* by Brandham (1971). This plant is a triploid, and all the chromosomes are normal except one of the L_1 chromosomes. This is a very short chromosome and is essentially isobrachial, with each arm about the length of the short arm of normal members of the L_1 chromosome. Brandham found no evidence of an interchange and has assumed this chromosome arose by a break in the long arm of a normal L_1 chromosome so that about three-quarters of the long arm broke away and was lost.

Brandham (1976) made an extensive survey of deletions in the Aloineae but found none in 1,543 plants of diploid aloes, diploid haworthias, diploid gasterias, and diploids of a few other genera. On the other hand, there were 14 (5.8%) individuals with deletions of 242 polyploid aloes, and 11 individuals (2.5%) of 449 polyploid haworthias. Of the 14 aloe deficiencies there were 3 (1.2%) different ones, and of the 11 haworthias there were 10 (2.2%) different ones. Most of the deletions in polyploid *Aloe* and *Haworthia* plants were in the long arms of the long chromosomes; one was in the short arm of a short chromosome of *A. elgonica;* and an entire short arm of a long chromosome of one hexaploid plant of *A. ciliaris* had been lost.

Brandham made a further study of *A. elgonica* (Brandham and Johnson 1977) and found several more deletions. Two plants had a deletion in the short arm of one short chromosome but was otherwise a perfectly normal tetraploid. Three plants similarly carried a deletion in the short arm of one short chromosome but also had twelve normal short chromosomes and therefore was an aneuploid ($2n$ = 29, 16 long and 13 short). One plant was a normal tetraploid ($2n$ = 28) except that it had a member of chromosome L_3 in which about one-third of the long arm had been lost. In an earlier study of the same region, Brandham found four plants which had short chromosomes with a piece of the short arm missing, three of which were aneuploids with 29 somatic chromosomes. There was also a plant with 28 chromosomes that had two such aberrant short chromosomes (cited in Brandham and Johnson 1977). That the deletions were restricted to polyploids suggests that they are lethal in diploids.

U-Type Bridges

The various basic steps of meiosis are fairly well understood as the result of a series of studies carried on in many laboratories over the last six or seven decades. In the zygotene substage of the first meiotic prophase homologous chromosomes synapse or pair side by side longitudinally; apparently soon thereafter each chromosome appears to be double or "split" into two chromatids, so that each configuration that is a bivalent in terms of chromosomes is a tetrad in terms of "half-chromosomes" or chromatids. At about this time breaks may occur in the chromatids, and these chromatids may exchange segments in such a way that one or several cross-shaped configurations can be seen in each bivalent. When a break occurs in one chromatid of one of the homologous chromosomes, a corresponding break occurs in exactly the same place in one of the chromatids of the other homologous chromosome. If, for simplicity, it is assumed that only one break occurs in each of the chromatids that break, these two chromatids together would comprise four broken pieces. A break in a chromatid will produce two broken ends, one on each side of the break. One end will be in the piece that is joined to the centromere and is the proximal end; the other end of that same break will be in the acentric segment and will be termed the distal end (Fig. 8.1*a*). Usually the proximal end of one broken chromatid will join the distal end of the other and the proximal end of the latter will join the distal end of the former. The result of these new unions will be a cross-shaped figure, or chiasma, that holds the two homologous chromatids together (Fig. 8.1*b*). At first anaphase the centromere in the bivalent starts to separate and pull apart. Soon each has progressed toward its own pole to the extent that the bivalent has come apart (Fig. 8.1*c*). At each pole there are two chromatids, but they are not identical throughout their lengths. The result of the chiasma and of the interchange of chromatid segments is very important because it produces genetic crossing over and considerably increases the chance of genetic variability.



8.1 Normal crossing-over: (*a*) chromosomes paired at pachytene with a break in one chromatid of each chromosome; (*b*) pachytene with union of the proximal end of each broken chromatid and the distal end of the other, forming a normal chiasma; (*c*) Anaphase I with the chromosomes separating to opposite poles.

In some plants occasionally this process occurs in a slightly different way. The two proximal ends may join together and the two distal ends may do likewise. The result is very different from the chiasma usually formed. If such an unusual union occurs, one long chromatid with two centromeres and one smaller chromatid with no centromere will be observed. Presumably a long dicentric U-shaped structure may be found at diplotene along with a smaller acentric U-shaped "fragment." At the ensuing Anaphase I the two centromeres pull apart toward the opposite poles as before. However, two nonsister chromatids are attached to one another at the former broken ends, forming the dicentric chromatid which then stretches from pole to pole. The acentric fragment cannot move to either pole since it has no organ of locomotion (centromere) and usually remains on the metaphase plate equidistant between the two poles. The exact pattern may vary to some extent depending on chiasmata that may also form in the bivalent. The picture as seen under the microscope involves a "chromatid bridge" that extends from one pole to the other and an acentric fragment that cannot move.

While one explanation for these U-type bridges and fragments has been presented here and is based on the theory of the related origin of U-type exchanges and chiasmata, it is not the only one theoretically possible and has not been proven by any means. An independent and unrelated origin may be the correct explanation, but the evidence for one or the other is not conclusive. The theory that errors in normal chiasma formation might produce U-type exchanges is simple and attractive and can be adopted tentatively. In most plants the study of U-type exchanges is handicapped by the fact that there is no way to identify and recognize any particular arm of a bivalent, but in rye Jones (1969) found a convenient marker in the localized neocentric activity of a specific locus at the end of the short arm of a submetacentric chromosome. This neocentric region could be recognized under the microscope as a small satellite. It was present at the end of the short arm of only one of the two homologous chromosomes of the specific bivalent, so two of the four chromatids of the tetrad could be recognized visually and had neocentric properties; the occurrence of a chiasma in that short arm could be recognized later in Anaphase I.

U-type bridges and accompanying fragments have been known for about 25 years, and references will be made to them again in this and subsequent chapters. For further discussions of them, see Matsuura (1950), Walters (1950), and Lewis and John (1966); see also Brandham (1970b).

Duplications

Duplications are chromosome aberrations in which a segment of a chromosome (not a whole chromosome) is present more than twice in a diploid organism. Duplicated segments may be small or large. If such a segment is large, it may loop out from the normal chromosome at pachytene; if small, it may be undetected. The duplicated piece may be found in a nonhomologous chromosome or in a homologous chromosome, where it may be present in the same arm or in

the other arm from the one in which the corresponding original segment is present; if in the same arm, it may be next to or separated from the original segment. If the original and the duplicated segments are together, the duplication is a "repeat" and the duplicated piece may be inserted in the same direction as the original segment or may be inverted. The duplication may also occur as a fragment with a centromere. Duplications may give disturbed genetic ratios and, if they are repeats, may produce a position effect.

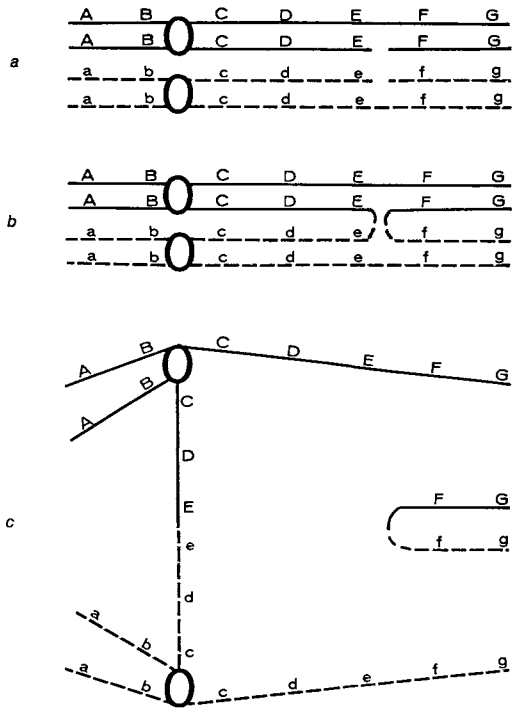
Duplications in plants are difficult to detect cytologically, especially in mitosis. A large duplication may increase the size of a chromosome greatly, resulting in a heteromorphic bivalent, but the same result could be obtained from a reciprocal translocation. If the duplication is small, it probably cannot be detected.

Duplications are best studied in organisms such as *Drosophila melanogaster* and species of *Sciara*. These flies have polytene chromosomes in their salivary glands and certain other tissues, and the chromosomes are characterized by cross bands or discs which are readily observed and identified. These bands occur in a definite pattern in all normal flies, and any deviation from this pattern indicates a type of chromosome aberration. If one or more bands are missing, a deletion or deficiency is indicated; an extra band shows a duplication. These polytene chromosomes have been of great significance in the interpretation and understanding of aberrations.

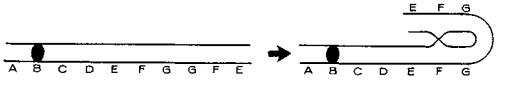
Since like all plants the Aloineae do not have polytene chromosomes, and since small duplications usually cannot be detected, duplications have not often been found in them; whether others do not exist or whether they are too easily overlooked cannot be stated at this time.

Deletions and duplications may be observed if they involve some striking feature such as satellites. Pinto-Lopes and Resende (1949) showed that the satellite at the end of one chromatid could be translocated to the end of the satellite of the sister chromatid so that the latter had two satellites in tandem. The result of this translocation was one deleted chromatid and a duplicated sister chromatid. Thus there was simultaneous deletion and duplication.

A fascinating duplication in an aloe hybrid has recently been reported by Brandham (1975). Heteromorphic chromosomes were present at meiosis and were similar to some of those described by the present authors; Brandham was able to trace their origin by meiotic studies, whereas the authors' heteromorphs were not studied at those divisions. Heteromorphs may arise through deletion or duplication, and it is not always clear which is their cause. Brandham's duplication, which was found in a triploid hybrid between *A. rauhii* ($2n = 14$) and *A. dawei* ($2n = 28$), was shown by careful meiotic studies to have resulted from a duplication. The writers' heteromorphic chromosomes could have had the same origin but not necessarily; they might have resulted from deletions or translocations. They were observed only in somatic cells and were probably duplications rather than deletions because they usually had a chromosome that was longer rather than one that was shorter than normal.



8.2 Origin of U-type bridges: (*a*) chromosomes paired as in Fig. 8.1*a*; (*b*) union of the two proximal broken ends and the two distal broken ends to form a U-shaped dicentric chromatid and a U-shaped fragment; (*c*) the resulting anaphase bridge and U-shaped fragment. (Modified from Brandham in *Chromosoma*, vol. 51)



8.3 Meiotic behavior of a univalent chromosome which carries a duplicated terminal segment in reversed position (a "reverse repeat"). (Reproduced from Brandham in *Chromosoma*, vol. 51)

Brandham's chromosomal segment was attached to the end of the long arm of a member of chromosome L_2 and was often relatively long — normally about 2.3 times the length of the short arm of that chromosome; but it varied to some extent and sometimes was no longer than the short arm. The ratio of the supernumerary segment to the short arm has a mean value of 2.2-2.4, with some higher values that are attributed to errors of measurement and some inconsistent lower ratios that are believed to result from erosion in some cells.

The supernumerary segment was found in the hybrid but was absent from the somatic cells of the two parents and was thought to have originated as an aberration at meiosis in one or the other. When *A. rauhii* was outcrossed with a number of other species, none of the offspring showed the aberration; but when *A. dawei* was crossed with other species, chromosome aberrations were always found in at least some of the offspring. These aberrations were interchanges or deficiencies, and Brandham therefore concluded that the supernumerary chromosomes arose during the meiotic divisions of *A. dawei*. When found, a rather clear lesion was observed between the supernumerary segment and the end of the long arm to which it was attached.

The suspected origin of this duplication involves the presence of U-type bridges, since these aberrations are present with small frequencies in both parents. If one chromosome has the sections ABoCDEFG (where o represents the centromere) and the other is abocdefg, and if the break occurs between the Ee and Ff regions (Fig. 8.2), the anaphase bridge would be ABoCDEedcoba and the U-shaped fragment would be GFfg. This bridge extends between the two poles; as the centromeres move toward the poles, it stretches and usually breaks. When any type of chromatin bridge is present, a failure to break may occur very rarely and a bridge may then be found connecting one pole of each of the two spindles at Anaphase II. The senior author (Riley 1948b) found this in a *Gasteria-Aloe* hybrid, but such a failure to break is rare. If the break occurred between segments d and e, two of the four cells found at the end of the second meiotic division would be normal (ABoCDEFG and abocdefg); one would have a duplication for Ee and Ff and a deficiency for Gg; and the fourth would lack segments Ee, Ff, and Gg and would be abocd. The original U-shaped fragment would probably stretch out into a rod and would be found at random in any one of the four cells. The ABoCDEFfe chromatid would have a duplication that would be a reverse repeat.

Since Brandham's plant with the supernumerary chromosome was a triploid, many univalent chromosomes were found at first meiotic metaphase; some of them represented the chromosome with the extra segment. In these univalents the terminal portion of the chromosome was often bent backward and appeared to be pairing with the adjacent segment of the chromosome, as in Figures 8.3 and 8.4. Therefore, the chromosome represented a reverse repeat type of duplication. If the segments of the normal chromosome were ABoCDEFG, this supernumerary chromosome might be ABoCDEFGgfe and a nonreverse repeat might be ABoCDEFGefg. In the reverse repeat the univalent would then pair back on itself. At anaphase in root-tip mitosis several

bridges were observed that had apparently been caused by errors in the division of the chromosome and subsequently broke at one or both lesions in the chromatids. If breaks occur at both, the chromosome segment representing the two extra parts of the supernumerary chromatids is lost and rounds up as a micronucleus.

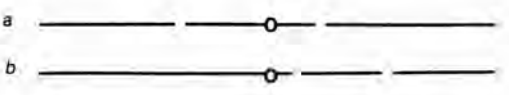
One hexaploid plant of *H. armstrongii* contained one chromosome that was longer than the normal chromosomes (Brandham 1976).

8.4 Univalent chromosomes at Metaphase I: four univalents with a terminal reverse repeat; at extreme right, a normal univalent. (Reproduced from Brandham in *Chromosoma*, vol. 51)

Inversions

An inversion is a chromosome aberration in which an intercalary piece of a chromosome breaks from the remainder of the chromosome and rotates 180° so that it is completely inverted with respect to the rest of the chromosome. Apparently a terminal segment cannot become inverted because the telomere cannot unite with the broken end of the chromosome at the place where the other end of the inverted segment was located. At least there is no real evidence for terminal inversions.

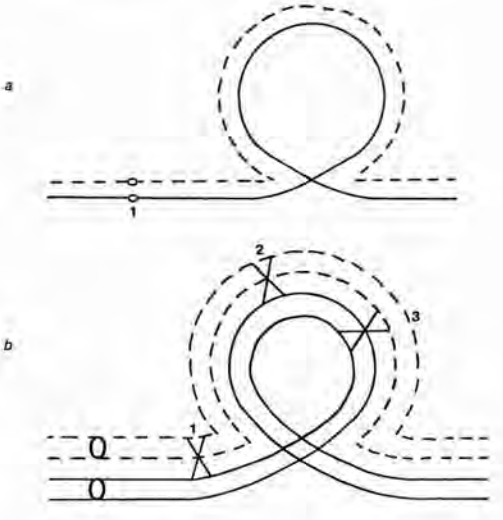
Since an inversion involves two chromosome breaks, there may be two types — pericentric and paracentric. The former occurs when the two breaks are on opposite sides of the centromere, and the latter is found when the breaks are on the same side of the centromere (Fig. 8.5).



8.5 Chromosome breaks that may form (*a*) pericentric and (*b*) paracentric inversions.

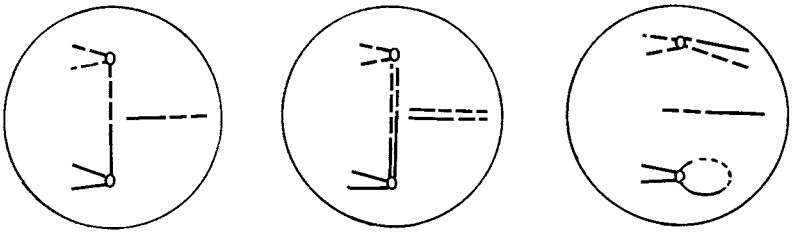
An inversion is best detected at pachytene or in the polytene chromosomes in some insects. At metaphase or anaphase of a somatic mitosis, as in the root tip of a plant, a chromosome that bears an inversion cannot be distinguished from the "normal" chromosome from which it was derived. Therefore, mitotic studies are useless for identifying or demonstrating inversions.

At meiosis inversions cannot be detected in organisms that are homozygous for the inverted segment, but in inversion heterozygotes a peculiar configuration can be seen at pachytene if the inverted piece is not too short. As McClintock (1931, 1933) demonstrated, a plant heterozygous for a long inverted segment has a "reverse loop" in pachytene because corresponding parts of two homologous chromosomes tend to synapse, even though the total picture of the bivalent is considerably distorted. If the inverted segment is small, apparently the two chromosomes cannot adjust to form the loop, and there may be some nonhomologous pachytene association or even some failure of pairing in the segment concerned.



8.6 Bridge and fragment formation from crossing-over within a paracentric inversion: (*a*) zygotene pairing between two homologs differing by a long paracentric inversion; (*b*) pachytene pairing in the tetrad stage showing three possible crossovers at 1, 2, and 3.

The interesting features of inversions are the occurrence of crossing-over within the inverted segment and the resulting patterns that are observed at Anaphase I, especially if the inversion is of the paracentric type. The two homologous chromosomes can be designated a and b. If they differ by a long paracentric inversion they will pair at zygotene in a characteristic loop, as shown in Figure 8.6*a*. The loop will consist of the two inverted segments. At the following pachytene stage each chromosome will consist of two chromatids that can be designated a^1 and a^2 and b^1 and b^2. The tetrad will still show the loop. Crossing-over may now occur between the two chromosomes. For purposes of illustration, crossing-over may take place



8.6*c* Anaphase I showing bridges, fragments, and loop if there is a crossover at 2 or 3 (*left*), at 2 and 3 (*center*), and at 1 and 2 or 1 and 3 (*right*).

between chromatids a^1 and b^1 at the place indicated in Figure 8.6*b* as 1, which is located between the centromere and the inversion loop. Other crossovers may be found at 2 as between a^1 and b^2 and at 3 as between a^2 and b^1. Other possible crossovers may also occur.

If one crossover should occur within the loop as at 2 or 3, the resulting Anaphase I configuration would be a chromatid bridge and fragment, as illustrated in the left-hand part of Figure 8.6*c*. A similar picture would be observed from crossing-over between chromatids a^1 and b^1 and between a^2 and b^2. If crossing-over took place simultaneously at 2 and 3, a double bridge and two fragments would be seen at Anaphase I, as shown in the central part of Figure 8.6*c*.

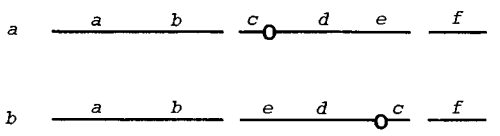
If a crossover occurs between the centromere and the inversion loop, as at 1 in Figure 8.6*b*, no bridge or fragment will be found, and the Anaphase I configuration will resemble the Anaphase I in organisms in which no inversion is present. However, if two crossovers occur — one within the loop and the other between the loop and the centromere — there will also be no Anaphase I bridge but there will be a chromatid loop at one of the poles, as in Figure 8.6*c*. If crossovers occur simultaneously at 1, 2, and 3, there will be a similar chromatid loop at each pole and there will be two fragments. If a loop is seen at Anaphase I, there will be a bridge and fragment at Anaphase II. As might be expected, the types with two bridges and fragments and two chromatid loops and fragments are much less frequent than are the Anaphase I figures with only one bridge and fragment or one chromatid loop and fragment.

The bridges and fragments found in Anaphase I as the result of crossing-over within an inversion are similar to those described earlier for U-type aberrations. In maize, bridges and fragments apparently result only from inversion crossovers; all the evidence obtained so far indicates that in the Aloineae inversion hybridity is much less common and most of the bridges and fragments result from U-type behavior or something related rather than from inversions. Brandham (1971) made a study of the frequency of the two types. Of 135 plants of *Aloe* six had inversion bridges and 73 had U-type bridges. Four *Astroloba* plants of six had U-type bridges, as did the only plant of *Chamaealoe*. Of 49 plants of *Gasteria* two had inversion bridges and 42 had U-type bridges. In *Haworthia* 39 plants had U-type aberrations and none had bridges arising from inversions. In all the plants with inversion bridges, U-type bridges are often but not always present in addition. Bridges and fragments have been found by numerous other

students of the Aloineae, but most of these studies did not involve measurement. Only with the recent meiotic studies of Brandham has an analysis of the types and an examination of their relative frequencies been made.

Inversions produce some interesting and important genetic effects. The more common type of inversion is the paracentric one, and these inversions form the most common type of chromosomal aberrations occurring in natural populations of plants in general. They act as crossover suppressors, because only noncrossover spores or gametes are usually recovered. Crossovers occur but do not appear in the offspring.

Pericentric inversions do not result in chromatid bridges, but they do produce duplication-deficiency chromosomes as the result of crossing-over within the inversion. The result is a reduction in fertility. Another possible effect is a change in the karyotype. If a chromosome is metacentric, one break near the centromere and another break near the distal end of the chromosome, followed by the 180° rotation of the inverted segment, will cause the previously median centromere to take up a new position nearer one end of the chromosome. The resulting chromosome will then be submetacentric or even possibly acrocentric (Fig. 8.7).



8.7 Change from a metacentric to an acrocentric chromosome as the result of a pericentric inversion: (*a*) metacentric chromosome with breaks near the centromere on one side and near the end on the other; (*b*) acrocentric chromosome that results from the inversion of the segment indicated in (*a*).

Two pericentric inversions have been found by Brandham (1974) in a population of *H. reinwardtii* var. *chalumnensis*. These are best studied at meiosis, but Brandham's plants have not flowered, so the evidence must come purely from mitotic observations. A pericentric inversion, if the breaks arise in the proper places, can cause an acrocentric chromosome to become metacentric and vice versa. Brandham's evidence for the inversions is of this nature, but, as he stated, the true situation cannot be demonstrated unless meiotic studies can be made.

Recently Brandham (1976) discussed the frequency of spontaneous inversions, both paracentric and pericentric, in populations of the various genera of the Aloineae. As he pointed out, inversion hybridity can be detected only by analyzing Anaphase I stages of meiosis. In his survey he found eight (6.5%) paracentric inversions in 123 plants of *Aloe* and two (4.2%) in 48 *Gasteria* plants but none in 72 plants of *Haworthia* or in 10 plants of other genera of the tribe. Pericentric inversions, however, are must less common. In *Aloe* he found only two (0.2%) pericentric inversions in 1,027 plants, and they were two different ones; in *Haworthia* he found seven (0.7%) in 1,046 plants, but only two different inversions; in 132 plants of *Gasteria* and in 29 plants of other genera he found no pericentric inversions.

In the Aloineae, pachytene stages are very difficult to study; the frequency of pericentric inversions has largely been estimated from a study of chromatid bridges and fragments at anaphase. One of the earliest studies was made by Kondo and Megata (1943), who showed several bridges and fragments. In *Haworthia tessellata* a bridge and fragment at Telophase I are nicely illustrated in their Figure 1*d*; in *Gasteria verrucosa* a bridge at late Anaphase I is shown in their Figures 2*e* and 2*f* and possibly in 2*c*. Bridges at Anaphase I are pictured in their Figures 4*b* and 4*c* from the intergeneric hybrid *Gasteria*

verrucosa X *Aloe variegata*. The illustrations are clear, but the discussion is in Japanese and incomprehensible to the present writers.

The senior author (Riley 1948b) found several bridges and fragments in a hybrid between *Gasteria* and *Aloe*. Most of them involved the long chromosomes, but in one mitotic cell a bridge connected two short chromosomes. The bridges were found in Anaphase I and Telophase I and also in Anaphase II. Some of the bridges in Anaphase II were perfectly normal bridges between the two sets of chromosomes at the two poles of the same spindle and were usually accompanied by a fragment that was clearly visible. Occasionally each of the two spindles of a dyad had a bridge. In a small number of cells an interesting bridge connected a late Anaphase II set of chromosomes on one spindle with a similar set of chromosomes on the other spindle. These bridges apparently were continuations of Anaphase I bridges. In some cells in Telophase I a bridge was formed between the two daughter nuclei but did not break. No cell wall normally forms across the equator of the first meiotic division in the Aloineae; therefore, in some cells the chromatid bridge does not break and persists through interphase. It connects the two spindles of the dyad at Anaphase II, extending from an anaphase set of chromosomes on one spindle to one on the other spindle (Riley 1948b, Figs. 13 and 14; Brandham 1977a). In this plant 47 Anaphase I cells were studied; six had a bridge between long chromosomes and one had a bridge between short ones. At Telophase I 29% of the cells had one or more bridges. At Anaphase II, of 23 cells one had a bridge on one spindle and three had a bridge on both. Whether the bridges were inversion or U-type bridges was not determined. In a second and phenotypically very different hybrid between *Gasteria* and *Aloe,* six of 87 cells at Anaphase I had a bridge and fragment involving long chromosomes and one cell had a bridge between short chromosomes and had an accompanying fragment. Of 38 cells in Anaphase II two had one bridge between the daughter nuclei on one spindle and one had a bridge connecting one nucleus on each spindle.

A meiotic survey of a number of species of *Gasteria* was reported by the senior author (Riley 1959d). One or more cells had bridges with fragments in *G. angustifolia* (4.2%), *G. carinata* (2.1%), *G. glabra* (24%), *G. mollis* (4.2%), *G. pseudonigricans* (3.8%), *G. sulcata* (4.9%), and *G. verrucosa* var. *asperrima* (2.0%). Seventeen other species had no bridges. Five hybrids also had no bridges and fragments, but four had one or more. Bridges were present in *G.* X*cheilophylla* (2.1%), *G. planifolia* X *G. sulcata* (3.2%), *G. sulcata* X *G. planifolia* (11.3%), and *G. verrucosa* X *G. brevifolia* (15.4%). A t test indicated no significant differences between the number of species and the number of hybrids possessing bridges; this suggests that while the number of bridges is rather large, inversions or U-type configurations apparently are not significant evolutionary factors in *Gasteria*. At that time U-type bridges were not understood; they were probably present but unrecognized.

Bridges and fragments were found in an apparently new and unnamed variety of *Haworthia limifolia* (Riley and Ma-

jumdar 1966b). This plant had 23 chromosomes and was a hypertriploid, with chromosomes L_2 and L_4 being represented four times and all the other chromosomes present thrice. Forty of 70 cells had a bridge plus one or two fragments at Anaphase I.

Interesting bridges have been reported by Darlington and Kefallinou (1957) and Brandham (1969a, b). These bridges have been interpreted differently in the two studies. They involve both translocations and inversions and will be discussed in the next chapter.

Paracentric and pericentric inversions have been studied chiefly in diploid organisms. Brandham (1971a, b) has found them in tetraploids and has analyzed some interesting configurations that have resulted from the extra sets of chromosomes. These reports are fascinating studies in cytogenetics, but inversions in diploids and polyploids probably have been of limited importance in the evolution of the Aloineae and therefore need not be considered in great detail here.

As mentioned earlier, and as McClintock (1931) showed, one or two crossovers within an inverted segment accompanied or not by one between the inversion and the centromere in a diploid heterozygote can result in four types of abnormalities in Anaphase I: a chromatid bridge and acentric fragment, a chromatid loop and a fragment, a double ridge and two fragments, and two loops and two fragments. The same types of aberrations can be found in tetraploids and triploids, but there are other complications that are interesting to consider. They depend on the formation of multivalents and on the number and position of chiasmata. In some tetraploids only bivalents might form, and the results would be the same as in diploid organisms. Because of the extra chromosomes, however, and because quadrivalents are frequent in tetraploids, some plants have configurations that are more complicated. Many of them are very rare. One of the less rare configurations, found in 7.31% of Brandham's (1977a) 875 Anaphase I cells of *Gasteria nigricans* var. *crassifolia,* is a W-shaped configuration; this can arise if the two centromeres that would form a bridge and go to opposite poles in a diploid should go to the same pole, as they sometimes do in a tetraploid. Another interesting aberration arises when three centromeres are linked by bridges to form tricentric chromosomes. Other configurations can also be seen at Anaphase I and will produce Anaphase II complications.

Pericentric inversions also occur in polyploid Aloineae, as Brandham (1977b) found in an artificial hybrid between *Aloe dawei* and *A. elgonica.* An inversion was present in one of the progeny of this cross, and it shifted the centromere of an acrocentric chromosome to a median position, as described above. Again the behavior of the inversion with crossing-over is the same as it is in a diploid plant; that is, two kinds of duplicate-deletion chromosomes are produced. In diploids the duplicate-deletion gametes do not survive, and about 50% sterility results; in tetraploids they frequently do survive, probably because the tetraploid possesses a full complement of genes in its normal set of chromosomes. The diploid does not have the normal set, only the duplicate-deficient set, and therefore does not have a complete genome.

Subchromatid Bridges

The bridges discussed so far involve whole chromatids, but in some plants subchromatid bridges have been reported. Vig (1970), in *Haworthia attenuata,* found that some cells of Anaphase I of meiosis had bridges with side arms. In two plants, out of 220 cells there were 54 cells with bridges, 29 with fragments, and three with both in Anaphase I. In second anaphase of meiosis, out of 54 cells there were three cells with bridges, two with fragments, and two with both. These aberrations are believed to be the result of subchromatid aberrations, although not necessarily half-chromatid aberrations.

Chapter Nine

TRANSLOCATIONS

Among the more common and certainly more interesting chromosomal aberrations or chromosomal structural changes are translocations (also called interchanges). A translocation is an aberration in which pieces of chromosomes are transferred to new positions in the karyotype by a process other than normal crossing over.

Translocations have frequently been classified into two types, simple and reciprocal. It was thought at one time that the simplest kind of translocation occurs when a piece of one chromosome breaks away and becomes attached to the end of another chromosome as a terminal translocation, but now it is known that this type of translocation cannot arise. The ends of a chromosomal fragment or of the chromosome from which it was broken could be said to be "open" ends; such an end is capable of reuniting with the end from which it was broken or of uniting with some other "open" end; but the normal end of a chromosome bears a telomere, and this end is not "open" and cannot unite with anything. Therefore, simple translocations of the terminal type do not exist.

Another type of simple translocation does exist, however, and is known as a shift. Such a translocation involves an interstitial chromosomal segment; it may be removed from its normal position and reinserted at a different place on the same arm or at an interstitial place in the other arm of the same chromosome or on a different chromosome. This type of aberration requires three breaks, one on each side of the broken piece and one in the chromosome arm at the place of insertion.

In a reciprocal translocation two pieces, one from each of two chromosomes, exchange places.

Normally translocations may be homozygous or heterozygous. Homozygous translocations behave no differently cytologically than do the chromosomes from which they were derived. If the translocated pieces differ considerably in size, the translocation chromosomes may be recognizably different from the original chromosomes; if they are of essentially the same size, the translocated plant and the original plant may not be differentiated cytologically. If many genes are known in the organism, the translocated plant may be separated from the original on the basis of its linkage groups.

Unless the translocated pieces are of unequal size, mitotic studies cannot be used to distinguish a translocation heterozygote from the original plant, but meiotic

9.1 Pairing of chromosomes in an individual heterozygous for a reciprocal translocation: (*a*) the two original chromosomes (*left*) and the two interchange chromosomes; (*b*) diplotene showing eight chromatids and one or two chiasmata in each arm; (*c*) the following metaphase (not showing chromatids) assuming complete terminalization of chiasmata. (Riley, 1948c)

studies can be so used. (Throughout this discussion, plants have been referred to rather than animals because translocations have been known much longer in plants and are less frequently found in animals.) Pachytene, diakinesis, and metaphase are excellent stages to use for a study of translocations in a heterozygote. Since synapsis is normally precisely part-by-part, the two original and the two translocation chromosomes arrange themselves in a cross-shaped figure. The place at which the chromosomes extend outward to form the arms of the cross is the place on the chromosomes where the translocation occurred. Thus it is usually possible to determine the place where the chromosomes interchanged pieces. Unless the arms of the cross are unusually short, as would be the case if one or both of the interchange places were near the end of a chromosome, one or more chiasmata can be expected to form in each arm. The chiasmata generally terminalize; when they have moved to the ends or nearly to the ends of the arms, the four chromosomes open out into a ring or circle (Figure 9.1). If chiasmata fail to form in one arm, a chain of four will be formed rather than a ring. These circles will be very apparent at diakinesis or metaphase. At metaphase the quadrivalent may be arranged on the equator as an open ring or as a figure eight. The open ring, usually referred to as the "adjacent arrangement," results in deficiency-duplication spores and therefore in inviable germ cells or spores in plants; the figure eight or "alternate" arrangement does not produce sterility because in this orientation alternate chromosomes go to the same pole. In some plants these arrangements seem to occur more or less at random; in others, as in *Oenothera,* only the alternate position is found. If only the adjacent arrangement were found in a given species, that species would soon die out. In some plants (as *Oenothera*) more than one interchange occurs and rings of 10, 12, and 14 chromosomes can result.

Reciprocal translocations probably have not been an important factor in evolution. In general gross structural changes are not the kind of variation that selection uses in evolution, nor in general do they provide phenotypic differences. Rarely (as in *Oenothera*) position effect may occur because of chromosomal breakage, but because it is not common it is not an important factor in the evolution of the plant kingdom. Although translocations may have been of evolutionary significance in building up isolating mechanisms, generally they were not important in evolution but were important in the history and development of cytogenetics, since in the early days they provided valuable information on chromosomal behavior.

Several reciprocal translocations have been reported in the Aloineae, including some of the centric fusion type. In a plant designated *Aloe gracilis* var. *minima,* Sato (1937) found eight long, one medium, and five short chromosomes instead of the usual eight long and six short found in most Aloineae. Sato believed that the karyotype suggested a translocation had occurred in which a piece of the distal end of a long chromosome (probably chromosome L_4) became attached to a short chromosome such as chromosome S_3, although he admitted it might have come from a fragmentation of chromosome L_4. Unfortunately no meiotic

studies corroborated this diagnosis. Normally there are four satellites in this species, and the fact that this plant had only three suggests one was lost from the end of an L_4 chromosome during the translocation process. Sato's description suggests that he thought a simple translocation had taken place. Much more likely, a fairly long piece of an L_4 chromosome exchanged reciprocally with a very short piece of the S_3 chromosome. In *Gasteria cheilophylla,* plant No. 1, one of the L_4 chromosomes had a satellite at each end. Sato (1937) interpreted it as the result of a translocation between the end of the long arm of an L_1 chromosome with its satellite and the normally nonsatellited end of the short arm of chromosome L_4, so this short arm now bore a satellite that had originally been attached at the end of the long arm of an L_1 chromosome. This plant probably should have been designated *G.* X*cheilophylla* Bak. and was probably a hybrid.

At about the same time Sato's results were published, Resende (1937b) reported several plants with apparent translocations. He studied four seedlings of *Aloe globuligemma* Pole Evans and observed that two roots of one of them had an unusual chromosomal situation: there were only six instead of eight normal long chromosomes. One of the mutated chromosomes was too long and the other was too short. The segment by which the size of the long chromosome was increased was exactly the same size as that by which the other chromosome was shortened. Resende interpreted this situation as an example of a simple translocation. About half the long arm of a long SAT-chromosome was translocated to another long chromosome at the end of its long arm. It must actually have been a reciprocal translocation involving a small piece of one chromosome.

Resende also (1937b) observed some indications of translocation in *Aloe schlechteri* Schoenl. (= *A. clavifolia* Burch., according to Jacobsen [1954]), based largely on the nature and number of satellites. These spontaneous mutations apparently involved an increase and a decrease in the size of satellites, a shortening of the threads connecting the satellites to the main body of the chromosomes, and the disappearance of satellites. Four seedlings — numbered 2, 3, 6, and 13 — were indistinguishable morphologically. In 16 roots of seedling 3 one of the four satellites was noticeably larger than the others; in seedling 2 one SAT-chromosome had a smaller satellite and shorter connecting thread than the three others; in plant 6 one satellite was attached to the short instead of the long arm of its chromosome; and in seedling 13 there were only three satellites, and one of them was very small and had a short connecting thread. These satellite situations corresponded to the number and size of the nucleoli. In seedlings 2 and 13 the satellites with the short threads could not be identified in metaphase. In seedling 2 one metaphase plate showed a chromosome with a larger satellite with a clearer connecting thread than usual, and this situation was also thought to have resulted from a translocation or perhaps an inversion.

Resende in the same paper suggested that in a species that normally has four satellites, the regular appearance of only three in any given individual results from a chromosomal mutation in germ cells and that a mutation of sim-

ilar nature took place in somatic tissue in *Gasteria maculata*. In seedling 6 it is believed that satellites with or without pieces of the chromosomes had been shifted by translocation from the end of the long arm to the end of the short. According to Resende, the translocation in *A. globuligemma* and those in the four seedlings of *A. schlechteri* illustrate the origin of asymmetry and also the origin of satellite types in the Aloineae through the appearance of mutations in the germ cells, followed by crossing and hybridization.

Sato (1942) found a translocation in a hybrid between *Aloe variegata* and *Gasteria verrucosa* var. *latifolia*. Part of the long arm of an L_2 chromosome seemed to be homologous with the short arm of another L_2 chromosome and with the long arm of an S_2 chromosome. Another translocation appeared to involve an L_2 and an S_2 chromosome in an *Aloe variegata* X *Gasteria "Gyu-zetu"* hybrid, and a medium-sized chromosome indicated a translocation between long and short chromosomes of *Gasteria gracilis* var. *minima*.

Evidences of translocations have been reported occasionally by several authors. Riley (1948a), in a triploid hybrid between *Gasteria sulcata* and *G. nigricans,* found one cell at metaphase that revealed a medium-sized chromosome which probably arose by reciprocal translocation or fragmentation. In some cells of the same plant a satellite was found at the end of the short arm of an S_1 chromosome (Riley, Hammack, and Majumdar 1968); it was thought to have arisen as a translocation in somatic tissue. In another cell of the same plant the two L_1 chromosomes were exceptionally long and the two L_4 chromosomes had large satellites. The origin of these chromosomes is obscure, but translocations may have been involved. On the other hand, in 25 diploid species and varieties of *Gasteria* there was no evidence of any translocations; no extra-long nor unusually short chromosomes were seen, and there were no chains or rings at first meiotic metaphase (Riley, unpublished).

Pinto-Lopes and Resende (1949) have found several translocations in *Haworthia*. They were interested in chromosome clumping, or "chromatic agglutination," as Resende (1941) has termed it. With insufficient evidence they assigned this as a cause of spontaneous gene mutations.

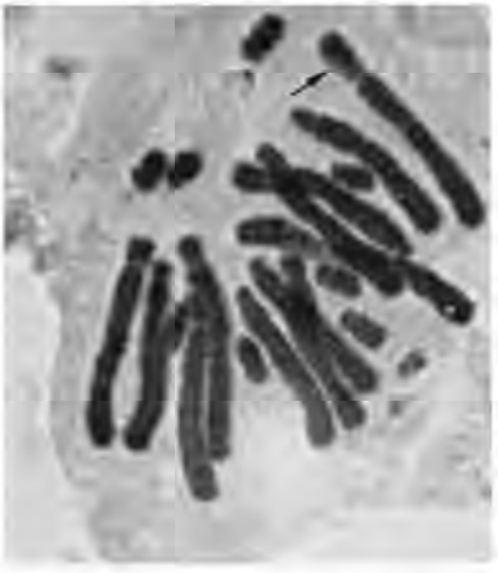
The translocations studied by Pinto-Lopes and Resende (1949) were primarily in the satellite and accompanying thread regions of the SAT-chromosomes (the "secondary olistherochromatic zones" of those authors). A frequently encountered translocation was one in which a satellite was deleted from one chromatid and translocated to the end of the satellite of the other chromatid, thus producing two satellites in tandem on one chromatid. Presumably such an aberration would be a simple translocation and was observed in both metaphase and anaphase of *Haworthia kewensis*. Apparently an identical translocation occurred between two half-chromatids and was shown at anaphase in a cell of *H. reinwardtii* var. *conspicua*. In one clear metaphase of *H. kewensis* a satellite of one chromatid broke off and attached to the satellite of the sister chromatid, while at the same time the broken end of the attachment

thread of the first satellite also became attached to the second satellite. Thus a proximal satellite was attached to both chromatids and a distal satellite was attached to it. A break in one thread connecting the proximal satellite to one chromatid will result in two satellites in tandem and therefore a duplication in one chromatid and a deletion in the other. A similar break followed by a joining of the distal satellite to the broken end of that thread so as to form a chain consisting of two chromatids and the two satellites, followed by a break between the satellites, will result in a reciprocal translocation of the two satellites. (By "proximal" is meant the satellite nearer the centromere and by "distal" the satellite farther from the centromere; these terms do not refer to the ends of the short and long arms of a chromosome, an erroneous usage by some cytologists.)

In *Haworthia* Mukerjee and Riley (1961) found some indication of transposed satellites. For example, in *H. planifolia* var. *planifolia* one pair of the long chromosomes had satellites at the ends of the short arms, and in an unidentified species of the Rigidae section satellites were present at the ends of the long arms of one pair of long chromosomes. In *H. attenuata* var. *caespitosa* two pairs of long chromosomes had satellites on the ends of the long arms, but one of the four chromosomes also had a satellite at the end of the short arm. This difference in the position of satellites in different species suggests that translocations have been occurring during the course of evolution. In 1976 Chinnappa and Semple reported a translocation involving satellites in two pairs of long chromosomes in one plant of *H. subfasciata*.

Evidences of typical reciprocal translocations have been found in a number of species by Riley and his coworkers. Some of them were somatic in origin and found in only one or a few cells of the root tip; others apparently arose in meiosis or in the postmeiotic microspore division. In a *Gasteria verrucosa* X *G. brevifolia* hybrid, for example (Riley 1959d), one cell of 176 in the microspore division had three long, one intermediate, and two short chromosomes instead of the normal complement of four long and three short ones, suggesting that a translocation had occurred at a preceding meiotic division. Riley and Majumdar (1968) found a plant of *H. fulva* in which one short chromosome was longer than usual and a long chromosome was shorter than usual (Fig. 9.2). The long chromosome involved was one of the L_4 pair, and the short chromosome was either an S_2 or an S_3 chromosome. This was not a single-cell event, for all the cells studied showed this translocation. Apparently a long piece of L_4 equivalent to about one-third of the long arm from the distal end interchanged with a shorter piece of the long arm of the short chromosome. The plant was a translocation heterozygote. Present evidence indicates that this ordinarily rather common type of interchange is rare in the Aloineae. However, it must be remembered that these translocations can be detected only if the two pieces involved in the interchange are noticeably different in size. A probable translocation is seen in *Astroloba pentagona* var. *spirella* where the short arm of one of the three L_1 chromosomes is

9.2 Translocation in *Haworthia fulva* resulting in a heteromorphic chromosome. (*Can.J.Genet.Cytol.*, vol. 10)



9.3 Heteromorphic chromosome in *Astroloba pentagona* var. *spiralis*. (*The Nucleus*, vol. 15)

much longer than usual (Majumdar 1968). This explanation is the simplest (Fig. 9.3).

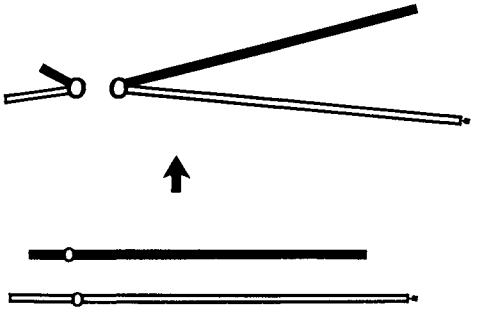
Other interchanges have been found. In *Aloe pubescens* (Brandham 1969b; Brandham and Johnson 1977) most of the short arm of an L_1 chromosome had interchanged with an extremely short piece of the long arm of an L_2 chromosome to produce an L_1 chromosome with a very small short arm and an L_2 chromosome with a long arm that was considerably elongated. This is the type of reciprocal translocation that at one time was regarded as a simple, terminal translocation. At meiosis a high percentage of quadrivalents was observed as well as some trivalents, bivalents, and univalents. Only 59% of the pollen appeared viable. In *Haworthia reinwardtii* var. *minor* the short arm of an L_3 chromosome and the long arm of an S_3 chromosome exchanged places, producing a long and a short interchange chromosome. A trivalent and univalent were observed in 23 cells at first metaphase, a bivalent and two univalents in five, and a quadrivalent in two. The pollen fertility was 61%. An interchange in *Gasteria candicans* (in addition to the E-type interchange) was indicated by the presence of occasional trivalents and univalents but was not analyzed.

A recent survey of a large number of plants by Brandham (1976) has revealed many new translocations. In diploid aloes 16 individuals had four different interchanges in 785 plants, and in gasterias two individuals in 132 had a total of two interchanges. There were 23 (3.0%) individuals with interchanges in 597 haworthia plants and eight were different specific interchanges. In all the other genera taken together there was one translocation in 29 plants. In polyploid aloes one individual in 242 plants had an interchange, but polyploid haworthias had 175 individuals with 38 different translocations in 449 plants. The latter figures are somewhat misleading; some populations with known interchanges, such as that in *H. reinwardtii* var. *chalumnensis*, were more extensively studied than others. Therefore Brandham felt that only the number of recognizably different translocations was significant. Brandham also showed that in *Haworthia* only seven different diploids in the Coarctatae section were interchange heterozygotes out of 176 plants and that they involved five different interchanges. In diploids of all the other sections taken together 16 interchange heterozygotes were found in 421 plants, and there were only three different interchanges. However, in the Coarctatae section, there were 175 interchange heterozygotes and 38 different translocations in 412 polyploid plants. In polyploids of all the other sections no interchanges were found in 37 plants.

A translocation between the long arm of one chromosome of the L_1 pair and that of a chromosome of the L_4 pair of a clone of *Aloe barbadensis* Mill. has been reported by Sapre (1977). This translocation is clearly seen at mitosis and results in quadrivalents in Metaphase I of meiosis. However, it is found in only some of the mitotic squashes and in only approximately 14 percent of the buds that were studied. Sapre believes that the interchange occurred when a new sucker was formed and resulted in the anomaly in only some of the cells in both the root and the shoot systems.

Centric Fusion Translocations

Several translocations of a type known as centric fusions have been found in various species of *Haworthia* and *Aloe*. These are interesting and of considerable importance in some plants and animals in the evolution of the karyotype. A centric fusion is a reciprocal translocation involving two or more acrocentric chromosomes. If two are involved, the translocation may be termed a simple centric fusion; if three or more acrocentric chromosomes are involved, compound centric fusion might be more appropriate. In a simple centric fusion a break occurs near the centromere in the long arm of one chromosome and near the centromere in the short arm of another (Fig. 9.4). Reunions of the broken arms occur in such a way that the two long arms and one centromere form a long metacentric chromosome and the short arms and the other centromere form a short metacentric chromosome. There is no fusion of centromeres, although the effect is as if there were. Robertson (1916), who referred to fusion, characterizes such V-shaped chromosomes as "derived in many cases from the linkage proximally of two non-homologous rods." Speculating on the origin of the V, he mentions two possibilities: "Whether the chromosome in the ancestral species was a V or two rods, I cannot say. If the ancestral chromosome was a V, then a break has occurred at some time and this break has been handed down generation after generation. On the other hand, if a fusion has occurred at some time, then the fusion condition has been handed down." Swanson (1957), discussing changes in form and relative size of chromosomes, states: "In animals the centric-fusion type of translocation appears to be a particularly prevalent mode of chromosomal change, but in plants most well analyzed translocations involve a part of, rather than a whole, arm."



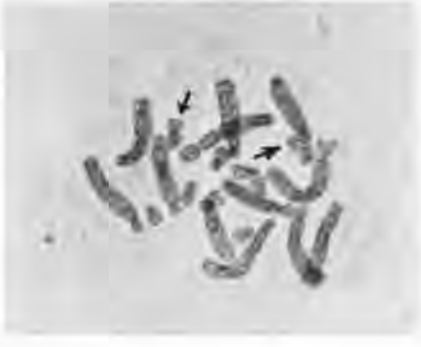
9.4 Diagram showing origin of centric fusion chromosomes. (*Can.J.Genet.Cytol.*, vol. 9)

Centric fusion translocations have been described fully by White (1954). He largely had *Drosophila* in mind and suggested that the short arm of the chromosome might be entirely heterochromatic, as is the short arm of *Drosophila* chromosome IV. If the small metacentric chromosome is largely inert genetically — being composed of two short arms that are entirely or almost entirely heterochromatic — it may readily be lost without any deleterious effect on the viability of the organism, since heterochromatin apparently has few if any genes. The effect of this loss would be to reduce the number of centromeres and chromosomes and thus change the chromosome number and the karyotype of the organism without affecting its viability.

Several translocations considered to be centric fusions were described in *Haworthia* by Riley and his coworkers. In 1967 Riley, Majumdar, and Hammack showed that in two plants of *H. attenuata* breaks had apparently occurred in the short arm of one acrocentric chromosome and in the long arm of another. In an unnamed variety there was a long metacentric and a short V-shaped chromosome that were different from any of the chromosomes of the normal complement. At the same time one normal L_1 and one normal L_2 chromosome were missing. Apparently an L_1 and an L_2 chromosome were involved in the interchange. The large metacentric chromosome had two long arms essentially

9.5 Chromosomes resulting from centric fusion in a variety of *Haworthia attenuata*.

9.6 Anaphase of root-tip mitosis in a plant with centric fusion, *Haworthia attenuata* var. nov. (*Can.J. Genet.Cytol.*, vol. 9)



9.7 Root-tip metaphase showing two small metacentric chromosomes (*arrows*) resulting from centric fusion of two short chromosomes. (*The Nucleus*, vol. 11)

the same as the long arms of the L_1 and L_2 chromosomes. In the short translocation chromosome the centromere was submetacentric (Fig. 9.5). One arm was just the same size as the short arm of L_1 and the other arm was the same size as the short arm of L_2 — further indication that the two chromosomes involved were L_1 and L_2. Measurements supported the theory that this was a centric fusion translocation. However, it differed from White's example in that the short arms of the chromosomes apparently were not heterochromatic and the short translocation chromosome therefore was not lost. The number of chromosomes or, better, the number of centromeres was the same as in a normal plant. The short translocation chromosome could possibly be mistaken for one of the normal short chromosomes, especially since it was not strictly metacentric but was somewhat larger; the short arm of chromosome L_1 was considerably longer than the long arms of any of the short chromosomes. Figure 9.6 shows two long metacentric chromosomes going to opposite poles at mitosis. In another plant of the same species an identical centric fusion type was found. This plant was also of an unnamed variety but appears taxonomically to be slightly different from the first. In a plant of *Gasteria sulcata* x *G. nigricans* one cell had two small metacentric chromosomes, one much smaller than the other; they may have arisen by a translocation in a somatic cell between S_2 and S_3 chromosomes. If they interchanged at the centromere, the large translocation probably consisted of the two long arms and the small one of the two short arms of these short chromosomes (Fig. 9.7) (Riley, Hammack, and Majumdar 1968).

Translocations resembling the simple centric fusion type were observed in some other plants of *Haworthia*. One appeared in a triploid specimen of *H. limifolia* var. *schuldteana* (Riley, Majumdar, and Hammack 1966, 1969). Again the centric fusion chromosomes were L_1 and L_2, and a long metacentric chromosome and a short chromosome with a submedian centromere were found. In 84 of 114 cells in first meiotic metaphase there was pairing between the long metacentric translocation chromosome and one or two of the normal homologous chromosomes (Fig. 9.8). In some cells both arms of the metacentric chromosome were tied by chiasmata to a normal homologous arm; in some cells only one was so paired; and in 30 cells there was no pairing with the translocation chromosome. Since this plant is a triploid, metaphase configurations involving the long metacentric chromosome could be quite complicated. In all cells the short translocation chromosome, consisting of the short arms of the L_1 and L_2 chromosomes, were never paired with the short arms of the normal chromosomes. Perhaps the reason is that the short arms of the chromosomes are too short to form chiasmata. Not all plants of this variety bear the translocation; Resende and Viveiros (1948) described a plant that did not.

Another apparent centric fusion type of translocation was observed in a tetraploid plant of *H. glauca* (Riley and Majumdar 1968). This plant was phenotypically normal in every respect except that it had smaller leaves than would be expected. Again an L_1 and an L_2 chromosome were involved in the translocation; only three of each type could be observed in the root-tip metaphase, whereas there were

TABLE 9.1 Lengths of Arms of Double Centric Fusion Chromosomes of *Haworthia glauca* var. *glauca*

	Three Normal Chromosomes		
Arm	L_1	L_3	S_3
Long arm	10.0 μm	9.6 μm	1.6 μm
Short arm	2.8 μm	1.6 μm	0.8 μm

	Metacentric and Acrocentric Chromosomes		
	Long	Medium	Short
Arm	Metacentric	Acrocentric	Metacentric
One arm	10.0 μm (L_{1L})*	2.8 μm (L_{1S})	1.6 μm (L_{3S})
Other arm	9.6 μm (L_{3L})	0.8 μm (S_{3S})	1.6 μm (S_{3L})

*The arms of the original chromosomes are indicated in parentheses: L_{1L} = long arm of chromosome L_1; L_{1S} = short arm of chromosome L_1; etc.

four each of the L_3, L_4, and short chromosomes (Fig. 9.9). Another translocation between two long chromosomes was observed in the triploid *H. reinwardtii* var. *chalumnensis*. Chromosomes L_1 and L_4 were involved, and again the short translocation chromosome persisted and was not eliminated. Chromosomes L_2 and L_3 were present three times, but L_1 and L_4 were present only twice. This is good evidence that L_1 and L_4 were involved in the translocation and was confirmed by measurements of the arms of the short translocation chromosome, which can be readily identified by the fact that it is longer than any of the normal short chromosomes since the short arm of L_1 is measurably longer than the long arm of any of the short chromosomes. In the diploid plants with the translocation there were satellites at the ends of the long arms of L_1. In this plant both the L_1 and L_4 chromosomes bore satellites on the long arms; since the long metacentric translocation chromosome consisted of the long arms of each, it carried satellites at the end of each arm (Fig. 9.10).

One plant was rather amazing because it apparently bore two centric fusion translocations (Fig. 9.11). It was a typical form of *H. glauca* var. *glauca* but differed from the one just discussed in having leaves of normal size rather than small ones. Since it was perfectly normal phenotypically, apparently neither the translocation nor the fact that it was a tetraploid was of any evolutionary significance. Resende (1937a) had previously found a typical plant of this species that was a diploid without any mention of a translocation. Both plants of *H. glauca* reported here were tetraploids. The one with the double translocation showed interchanges involving chromosomes L_1, L_3, and S_3. The lengths of these arms (Fig. 9.11) were as shown in Table 9.1. The interchanges resulted in three translocation chromosomes — a long metacentric, an intermediate acrocentric, and a short metacentric. Measurements of the arms of these chromosomes also are shown in Table 9.1 and illustrated in Figure 9.12.

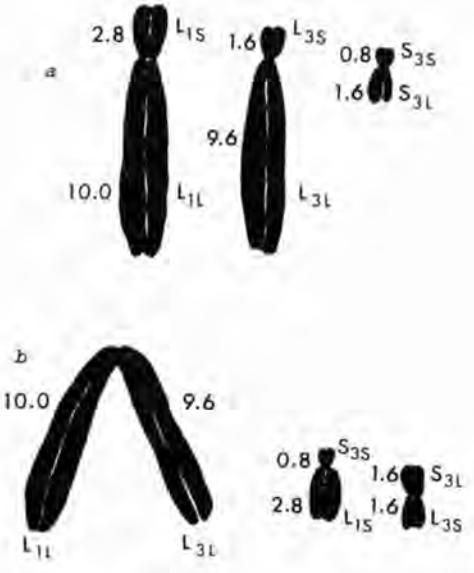
It seems that three chromosomes were involved in two interchanges. Whether the interchanges occurred simultaneously or at different times cannot be ascertained. Ap-

9.8 Chromosomes of *Haworthia limifolia* var. *schuldteana* which resulted from a centric fusion type of translocation.

9.9 Chromosomes from a centric fusion of *Haworthia glauca;* arrows point to translocation chromosomes.

9.10 Centric fusion in satellited chromosomes of *Haworthia reinwardtii* var. *chalumnensis;* arrows point to translocation chromosomes.

9.11 Chromosomes resulting from a double centric fusion in *Haworthia glauca* var. *glauca*; arrows point to long metacentric, short metacentric, and short acrocentric translocation chromosomes.



9.12 Diagram illustrating the probable origin of double centric fusion chromosomes: (*a*) normal chromosomes; (*b*) centric fusion chromosomes.

parently both of them took place at the centromeres, so in that respect they are typical centric fusions. So far as one can tell, the long and short arms of all the chromosomes are euchromatic; therefore, all three translocation chromosomes are euchromatic and are not eliminated as they are in many other organisms. In spite of the interchanges 72% of the pollen was fertile; this percentage seems rather high for a tetraploid with two interchanges. Two capsules were formed on this plant during the period of observation and bore 11 and 12 seeds respectively. Four of the first and two of the second germinated. The seeds must have arisen by self-fertilization since they formed while the plant was isolated from all others of its genus.

A summary of these translocations showing the chromosomes involved is given in Table 9.2.

Seven plants involving eight centric fusion translocations were found by the present writers and their associates. Three were diploids, two were triploids, and two were tetraploids. Chromosomes L_1 and L_2 were involved in two diploids and one triploid; L_1 and L_4 were involved in one triploid and one tetraploid, and L_1 and L_3 along with S_3 in the other tetraploid. Why chromosome L_1 should be involved in so many translocations is difficult to understand, but careful measurements of the long and short arms of all seven chromosome types and of the arms of the long and short translocation chromosomes dispel any possible doubts on this peculiar situation.

In 1973, Brandham collected five plants of *Aloe rabaiensis* and observed (Brandham and Johnson 1977) that one of them had a centric fusion type of translocation between chromosomes L_2 and L_3 that was similar to those previously discussed for *Haworthia*. It is especially interesting because it is the first translocation of the centric fusion type discovered in *Aloe*. Brandham subsequently restudied the site from plants sent to him at Kew by M. A. Hanid. Of 66 cuttings he received, 57 rooted satisfactorily and were studied. Fifty of these were normal diploids and the other seven were centric fusion interchange heterozygotes. No interchange homozygotes were found, a situation similar to that found in earlier studies of *Haworthia*.

Sharma and Mallick (1965) recorded an interesting situation in a plant they designated *Aloe pringeli* Jacobsen. (It is not found in Jacobsen [1960], however.) They described and depicted two short chromosomes with median centromeres and named them type E and type J. Type E consisted of "medium sized chromosomes with median primary constrictions," and type J comprised one "very short chromosome with a median primary constriction." In *Aloe pringeli* "both E and J type chromosomes possess median constrictions and are non-homologous." Sharma and Mallick believed this species was "possibly a structural hybrid." They considered that a segmental translocation must have taken place between the two short chromosome types so that a "portion of the long arm" of a short chromosome with a nearly submedian primary constriction was "translocated to the short arm" of a similar chromosome. It is difficult to determine whether Sharma and Mallick believed this to be a simple translocation or a reciprocal translocation, but there is every reason to believe that it is an example of a centric fusion translocation between two short chro-

TABLE 9.2 Seven Translocations Found in *Haworthia*

Species	2*n* Chromosome Number	Type of Translocation	Chromosomes Involved
H. fulva	2*x*	plain reciprocal	L_4 and S_2 or S_3
H. attenuata	2*x*	centric fusion	L_1 and L_2
H. attenuata var.	2*x*	centric fusion	L_1 and L_2
H. limifolia var. *schuldteana*	3*x*	centric fusion	L_1 and L_2
H. reinwardtii var. *chalumnensis*	3*x*	centric fusion	L_1 and L_4
H. glauca [small-leaved form]	4*x*	centric fusion	L_1 and L_4
H. glauca [typical form]	4*x*	double centric fusion	L_1, L_3, and S_3

mosomes. The two arms of the longer of the two short chromosomes with median centromeres (type E) are about the same length as the long arm of the typical short chromosomes, while the two arms of the shorter of the two chromosomes (type J) are the same length as the short arms of typical short chromosomes. These size relationships fulfill all the requirements of a centric fusion translocation except that, as in all other apparent centric fusions in the Aloineae, the shorter of the two metacentric chromosomes is not lost as it is in centric fusion translocations in animals.

Brandham (1976) has reported the results of an extensive study of interchanges in the Aloineae. He studied 51 breaks at mitosis and found that nine were of the Robertsonian (centric fusion) type except that the small metacentric chromosome was not lost — an observation the present authors had also made, as mentioned previously. These breaks in Brandham's material, like those of the present writers (Riley and Majumdar 1968) were at the centromeres; but Brandham also found other breaks at the centromeres that did not produce centric fusion type chromosomes. In all of 102 breaks, 30 were at the centromeres and only 72 were along the rest of the chromosomes, which he considers "a large departure from randomness in favour of centromere breaks." He further commented that these observations of spontaneous breaks are different from the breaks that result from ionizing radiations; the latter are only rarely found at the centromere or even near it, as reported by Sjodin (1971) for *Vicia faba*.

Riley and Majumdar (1968) listed seven plants of *Haworthia* that had interchanges (Table 9.2). One, *H. fulva*, had a translocation that was not of the Robertsonian type and had breaks that were almost certainly not at the centromeres; the other six plants (seven breaks) had interchanges of the centric fusion type and therefore had breaks at the centromeres. The writers pointed out that even in polyploids this short metacentric chromosome was not missing, though the loss would not be as severe as it would in a diploid.

Riley and Hoff (1958) studied the problem of localized spontaneous and induced chromosome breakage in *Tulbaghia*

violacea, an African member of the Amaryllidaceae or Liliaceae (according to the taxonomist), because of several observations other investigators had made on other plants. For example, Levan and Lotfy (1950) found that *Vicia faba* seeds soaked in tap water 24 hours before they were allowed to germinate had large acentric fragments in numerous anaphase figures. The frequency of these aberrations decreased daily until the seventh day, when they almost disappeared. Several chromosomes seemed to be involved and the breaks always occurred at the pronounced secondary constrictions near the centromeres. Since these breaks did not arise in seedlings that had not been soaked, Levan and Lotfy attributed the breakage to anaerobic conditions during the soaking.

Sharma and Bhattacharyya (1956) found numerous spontaneous symmetrical breaks in *Vicia sativa,* which they also believed arose at the secondary constrictions. These breaks were present chiefly in young roots and were found whether or not the seeds were presoaked. Oehlkers (1953) found that in *Vicia faba* many of the spontaneous breaks were observed at the secondary constriction (SAT zone) and that the spontaneous breaks in that region were more frequent than the induced breaks. Emsweller (1947) found that in the D and E chromosomes of the Easter lily (*Lilium longiflorum*) breaks occurred from the pressure of the cover glass during the preparation of root-tip squashes and that they occurred at the secondary constrictions. He considered these constrictions points of relative weakness.

These interesting observations of the weakness of the secondary constriction suggested that a similar study might be made on *Tulbaghia* chromosomes, since there is a large prominent secondary constriction in one of the members of the haploid set of chromosomes (Riley and Hoff 1960). Four pairs of these chromosomes are metacentric; one pair has subterminal and one pair has submedian centromeres. In both members of this last pair a secondary constriction is located in the long arm near the centromere. They are readily seen in the microspore division but not so readily in the root tips unless the chromosomes are contracted by cold treatment, as discussed in Chapter 4. No additional constrictions except the centromere (primary constriction) and secondary constriction were revealed by the cold treatment. Root tips studied one or two days after soaking had a relatively high frequency of breaks at these two constrictions compared to the remainder of the chromosome, and the breaks at the secondary constriction were about five times as numerous as those at the centromere. The frequency of all breaks diminished daily until about eight or nine days, when they disappeared except for an occasional fragment broken off at a region other than a constriction. X-rays caused a considerable increase in chromosome breaks at the centromere and secondary constriction as well as in the rest of the chromosome. Radiation-induced breaks were about equally frequent in the two constrictions and, considering the relative lengths of the constrictions and the other parts of the chromosomes, were more frequent in the constricted regions than in the other parts of the chromosomes. Radiation (162.5r), 0.001M pyrogallol, 0.001M resorcinol, and

0.001M phloroglucinol had about the same effect. In general it seems as if these two constricted regions are regions of weakness.

The tendency of chromosomes to break near the centromeres followed by translocation to produce centric fusion chromosomes may have two important evolutionary consequences: (1) The small interchange chromosome may be lost and the chromosome number may therefore be reduced. Apparently this is a widespread occurrence among organisms in general and is perhaps universal among animals, but it has never been found in the Aloineae. (2) The formation of metacentric chromosomes alters the karyotype because all typical chromosomes of this tribe are acrocentric.

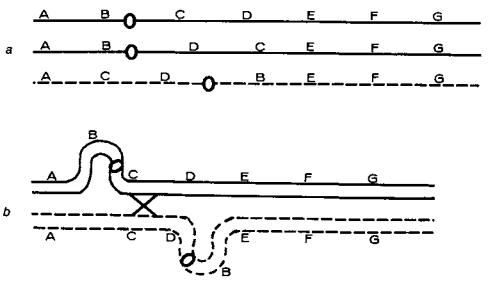
Either of these events would, if perpetuated, profoundly affect the course of evolution in the tribe. However, the first does not occur in the Aloineae, so no deviation from the basic chromosome number is ever found except for an occasional aneuploid. The second phenomenon is not common, and in none of the centric fusion plants that have been found has the condition become homozygous. Brandham (1976) found that all the tetraploid and triploid plants of *H. reinwardtii* var. *chalumnensis* he studied were inversion or exchange heterozygotes and that natural selection favors the exchange heterozygote, although it does not do so in some other *Haworthia* species. He pointed out that in all other members of the tribe studied, asymmetrical structural changes appear to be selected against.

E-Type Bridges

An interesting translocation involving E-type bridges was reported by Brandham (1969a) a few years ago. It was found in 20 of 163 taxa studied in the Aloineae, and its occurrence is believed to show a high degree of interspecific hybridity. Apparently, according to Brandham, the bridges had been observed previously by other cytogeneticists but not interpreted correctly. Sato (1942) found bridges in *Aloe* X *Gasteria* hybrids which he interpreted as abnormal separations; but, as Brandham pointed out, several of his photomicrographs and drawings show clearly that they are the same as E-type bridges. Darlington and Kefallinou (1957) illustrated them clearly but considered them to have been caused by a misdivision of the centromere.

The E-type bridge comprises two acrocentric chromatids that are connected at the centromeres by a moderately long thin thread of chromatin and move to the opposite poles of the spindle. Attached to the bridge between these two centromeres are one long arm and one short arm (Fig. 9.13). The three long arms as they usually appear frequently form a well-defined letter E. There are three main parts to the bridge: the central region that joins the long and short arms in the central part of the bridge, and two terminal sections that join these long and short arms to the respective centromeres at either end of the bridge. Exactly how they are formed is not known. Various possible explanations for their origin have been considered by Brandham. One assumes that the centromeres are greatly elongated and end at the long and short arms to-

9.13 E-type bridges and fragments: (*a*) a typical E-type bridge in *Gasteria schweickerdtiana* with three long arms and one short arm forming the E and one long fragment; (*b*) *G. candicans* showing a bridge formed from an exchange in a short chromosome. B: bridge; C: stretched centromere; F: fragment. (From Brandham in *Chromosoma*, vol. 27)

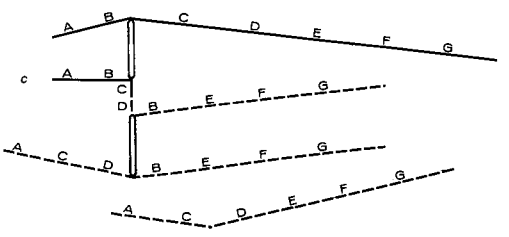


9.14 The origin of E-type bridges: (*a*) in the original chromosome a break has occurred between C and the centromere, and another between D and E; the segment has inverted to form the second chromosome; in it breaks have occurred between A and B and between C and E, and this segment has also inverted to form a third chromosome; (*b*) pairing at pachytene; the X represents a crossover.

ward the center of the bridge. Between the centromeres is a fine thread of chromatin which is usually stretched and later breaks. It is part of two chromatids. At the outer end of each elongated centromere is a complete chromatid. These two complete chromatids have the same chromosomal segments but differ in the arrangement of the segments, so one has a longer long arm and a shorter short arm than the other. At the inner end of one centromere is a short arm, and it is the same as the short arm of the whole chromatid that goes to that pole. Continuous with this short arm is a small segment of the same chromatid. Translocated to this segment is a piece of the other chromatid which extends to the inner end of the other centromere. Attached to it and extending out from the centromere is the long arm of that chromatid. At the outer end of that centromere is the other whole chromatid. A fragment is also present that consists of part of the short arm of one chromatid and part of the long arm of the other chromatid. The piece of chromatin forming the central part of the bridge may be very small so that the short and long arms that protrude from the bridge may appear to be in contact. The three long arms are usually considerably longer than the short arm, so the bridge usually has the appearance of an irregular letter E.

The simplest explanation Brandham has proposed for the origin of the E-type bridges involves two successive inversions in the same part of the chromosome. If the original chromosome is ABoCDEFG and an inversion of the CD segment occurred, the resulting inversion chromosome would be ABoDCEFG. If in the new chromosome the BoDC segment then became inverted, the resulting chromosome would be ACDoBEFG (Fig. 9.14*a*) and would also be an inversion chromosome. It should be noted that the CD segment is restored to its former orientation but is now in the short arm rather than in the long. It should also be noted that the short arm is longer and the long arm is shorter than previously; that when the new chromosome is combined with its normal homologue they form a heteromorphic bivalent; and that the acrocentric chromosome tends to become metacentric or at least less acrocentric, depending on the relative lengths of the pieces of short and long arms involved. Since the CD segments are identical in the inverted and normal chromatids, they pair or tend to pair with one another at zygotene (Fig. 9.14*b*). The B segments cannot usually pair because the chromosome cannot twist around for them to come together, so they must bulge out in each chromosome as in a deletion or duplication. Also, because of their position the centromere regions do not pair together. If the B segment were actually longer than the CD segment, the B segments rather than the CD segments would tend to pair. Since the CD segments are paired, a certain amount of chiasma formation and crossing-over takes place between them. The CD piece of the long arm and the CD piece of the short arm are identical, so they pair and cross over. Because of the two successive inversions, the CD segment is not in an inverted position, so no inversion loops are present at that place. It must be realized that these two chromosomes might be exactly the same in size and not heteromorphic with respect to total length but are heteromorphic when the relative lengths

of the short and long arms rather than of the whole chromosomes are considered. Heteromorphic chromosome pairs are not rare in *Haworthia* and have been reported by the present writers as well as by Brandham and others. According to this ingenious explanation of Brandham, the two centromeres are greatly elongated so that the two short arms on one side of the bridge may be well separated in space, as may also the two long arms of the other chromosome (Fig. 9.14*c*). The fragment is naturally acentric and generally lies on the equator.



9.14*c* First anaphase with an E-type bridge and fragment; highly diagrammatic; the relative sizes of the long and short arms will depend on the size of the inversion and the place of crossing-over. (Based on diagrams by Brandham in *Chromosoma*, vol. 27)

In most of his studies Brandham found that the large chromosomes were responsible for the E, but occasionally the small chromosomes were involved, as in *Gasteria schweicherdtiana*. It might also be pointed out, as Brandham has done, that only organisms with acrocentric chromosomes can show E-type bridges. Plants with metacentric chromosomes probably can produce E-type bridges; but these bridges cannot be recognized and identified as such since similar configurations can be produced by inversions, U-type exchanges, and E-type exchanges in metacentric chromosomes.

It is extremely interesting to note that the L_1 chromosome is heteromorphic and is capable of forming E-type bridges in all the plants studied by Brandham. Since this translocation was involved in all the writers' centric fusion translocations also, this feature is probably highly significant.

Recently Brandham (1973) reported five new translocations in three genera of the Aloineae. *Astroloba foliolosa* is a plant with 14 somatic chromosomes and behaves a little irregularly at meiosis, having a small percentage of U-type bridges and subchromatid bridges and a very high percentage of E-type bridges. Brandham considers these E-type aberrations an indication that the plant is of recent hybrid origin. Because it also has a few Metaphase I cells with some quadrivalents made up of the large chromosomes, Brandham believes it has an interchange although the interchanged segments must be of about equal size since they cannot be detected at mitosis.

In *Aloe gloveri*, a diploid, an interchange had occurred between the entire short arm of one member of chromosome L_3 and the entire short arm of one of the short chromosomes. The particular short chromosome could not be identified. At Metaphase I of meiosis these two aberrant chromosomes and their two normal homologues usually formed quadrivalents — either a ring or, less frequently, a chain of four.

Brandham (1973) also reported three translocations in *Haworthia*. In *H. browniana*, a diploid, two chromosomes of intermediate length were present but were not identical; one was submetacentric and the other was decidedly acrocentric. In this particular plant apparently a large part of the long arm of a member of chromosome L_1 interchanged with most of the long arm of one of the short chromosomes. Again there was a high percentage of quadrivalents, but the percentage of chain quadrivalents compared to rings of four was somewhat higher — almost certainly the result of a lower chiasma frequency. This same species had been studied previously by Resende and Da Franca (1946) and clearly had the same interchange, but Resende and Da Fran-

ca failed to recognize one of the aberrant chromosomes and therefore missed the correct interpretation. *H. umbraticola* var. *hilliana* is a classic example of a centric fusion type of interchange. The short arm of one member of chromosome L_3 and the long arm of one member of chromosome L_4 interchanged to produce a long metacentric chromosome composed of the long arms of each and a short, essentially metacentric chromosome comprising the short arms of each. Since the short chromosome consists of the two short arms of the two long chromosomes that possess the smallest short arms, it is so much like the six normal short chromosomes that it cannot be distinguished from them with certainty.

The other plant of *Haworthia* which Brandham (1973) reported in this paper was a triploid, *H. reinwardtii* var. *chalumnensis*. This variety had been studied previously by Snoad (1951a) and by Riley, Majumdar, and Hammack (1967). Snoad included it in his list of 49 species and varieties of *Haworthia* as a triploid but did not discuss it, and apparently his specimen did not include a translocation. The other authors also found it to be a triploid, but they also found it had a classic centric fusion resulting in a long metacentric chromosome and an additional acrocentric short chromosome a little longer than the normal short chromosomes. This was one of a series of three species of *Haworthia* with centric fusions first found by the senior author and his group. It was believed that the interchange occurred between chromosomes L_1 and L_4 and that the short translocation chromosome comprised the short arms of each. Since chromosome L_1 has the longest short arm of any of the short chromosomes, this translocation chromosome is longer than any of the normal short chromosomes. Brandham's plant of this species has an interchange, but he identified it as between chromosomes L_1 and L_3.

Brandham (1974) more recently published an extensive study on *H. reinwardtii* var. *chalumnensis* which included 145 triploid and tetraploid plants from eleven localities in an area west of East London, South Africa. These plants included some interchanges and represent the first published study of interchanges within a natural population. Twelve different spontaneous interchanges are described, and all but one was heterozygous only; two pericentric inversions were also found. Brandham's numerous interchanges form a complicated series involving chromosomes L_1 and L_3, L_1 and L_4, L_3 and L_4 (three different interchanges), L_2 and L_3, L_2 and L_2 (an unequal interchange between segments of the long arms of homologous chromosomes), L_1 and a short chromosome, L_2 and a short chromosome, L_3 and a short chromosome, L_4 and a short chromosome, and two short chromosomes.

Of the 145 plants of *H. reinwardtii* var. *chalumnensis* from the East London area, all had at least one aberration and several plants carried two or three. When the distribution of the various types throughout the area is considered, much information is obtained concerning the evolution of this variety. Since this variety is at the eastern edge of the range of the whole species, it is believed that the ancestral form of the variety should be at the western edge of the region of the variety. Here a tetraploid was found with the inversion but no obvious in-

terchanges, and the inversion was purely local. Apparently the taxon spread eastward from there and the most common interchange (L_1L_3) arose and occurs throughout the whole region. Subsequently all the other interchanges arose in various individuals of the taxon. One is apparently the oldest since it is the most widely distributed and is found in several sites of the central and western part of the area. The other interchanges must have arisen more recently since they are of very local distribution.

Several other points of interest developed from this study. One is that, although the interchanges were fairly numerous, there seemed to be no obvious position effect as is present in *Oenothera;* the external morphology varied little, if at all, from one interchange plant to another. Brandham pointed out that the triploids were morphologically identical with the tetraploids, showing that the morphology genes of the tetraploid are dominant over those of the unknown diploid parent or that the diploid and tetraploid parents are morphologically identical. Another point involves the nonrandomness of the spontaneous breaks. Early studies indicated that the L_1 chromosome of the Aloineae is more frequently involved in interchanges than random breakage would require. These newer data from *chalumnensis* indicate this is not so; among the four long chromosomes breakage is at random. Another interesting point is that centric fusion interchanges do not become established in homozygous condition in this tribe and therefore do not change the basic karyotype of the Aloineae. It seems there is selection against the interchange type and in favor of the original type, and therefore homozygotes do not arise or do not survive.

Isochromosomes

Occasionally metacentric chromosomes are found that are not the result of centric fusions. If the two arms are exactly the same length and are believed to be identical, the chromosome is an isochromosome. Apparently there is a misdivision of the centromere which splits transversely so that two homologous arms are attached to a half-centromere. They open out, subsequently forming a chromosome with two identical arms and a terminal centromere. In the Aloineae such isochromosomes have been found by Giles (1943) in a *Gasteria* species and by Brandham (1970a) in *Haworthia icosiphylla* Bak.

Chapter Ten

MISCELLANEOUS STUDIES

In earlier chapters the number of chromosomes in a plant, the behavior of chromosomes in cell division, the various possible anomalies of chromosome behavior that can occur, and the various types of chromosomal aberrations that are seen in different plants have been discussed and have been considered as possible factors in plant evolution. However, in many species factors other than chromosomal have been operating and methods other than those of classical cytology have been used in studying evolution. Some of those factors and methods will be considered here, including meiotic pairing, self-incompatibility, fertility, biochemical profiles as revealed by paper chromatography, leaf and pollen structure and cell structure in callus tissues as shown by the electron microscope, and the inheritance of leaf pigmentation as studied by the methods of classical genetics. Ecological and population studies have been interesting and important and will be discussed in Chapter 12.

Meiotic Pairing

Meiotic studies in the Aloineae have not been as numerous as karyotype analyses or determinations of chromosome number, but some studies have been made and provide some information on genomic relationships. The first reference to the chromosomes of first meiotic metaphase was by Taylor (1924) in *Gasteria verrucosa* (Mill.) Haw. and *G. cheilophylla* Bak. (or "intermediates between them"). He observed, as has been shown since, that the morphological differences among the chromosomes were the least evident at the first meiotic (heterotypic) division. Especially is this true of the three pairs of short chromosomes, all of which seem to have the same shape and size. Among the long chromosomes one bivalent appears at metaphase to be different from the other three pairs. It is undoubtedly chromosome L_1 since it has a longer short arm. Taylor also found that another member of the group of long chromosomes moved to the poles "considerably in advance of the rest," but it was not identified. In general, however, meiosis follows the conventional pattern of meiosis in most species of plants and animals.

Kondo and Megata's study (1943) of *Gasteria* showed less regularity of pairing at first meiotic metaphase. Their drawings of meiosis in *G. verrucosa* appeared to show

an occasional failure of the members of one pair of long homologues and/or of one pair of short ones to synapse with the resulting appearance of univalents on the metaphase plate. Those authors also showed apparent precocious separation of the members of one or two pairs of short chromosomes, and they depicted one cell in which one pair of long chromosomes had not undergone synapsis. In that plant 29 cells showed seven bivalents, 30 had six bivalents and two univalents, and 11 had five bivalents and four univalents. In *Aloe variegata* X *Gasteria verrucosa* there were seven cells with seven bivalents, 36 with six bivalents and two univalents, 17 with five bivalents and four univalents, and four with four bivalents and six univalents. In the reciprocal plant, *G. verrucosa* X *A. variegata,* six cells had seven bivalents, 40 had six bivalents and two univalents, 25 had five bivalents and four univalents, five had four bivalents and six univalents, and one cell had three bivalents and eight univalents.

In one study of species and hybrids of *Gasteria* (Riley 1959d), pairing seemed to be much more regular in both groups than Kondo and Megata (1943) had reported. Among the species in that study 13 had only regular pairing (seven bivalents); the number of cells counted varied from 38 to 129 in the different species. Ten other species varied from only two pairs and two univalents among the short chromosomes to six univalents. The long chromosomes showed perfectly regular pairing except that in two cells of *G. obtusifolia* one long bivalent was missing and in one cell of *G. fasciata* one pair of long chromosomes was missing and there was an extra short chromosome.

Among putative hybrids, as might be expected, pairing was less regular. Only *G. margaritifera* showed regular pairing, and in that plant only 26 cells were studied. Of eight hybrids studied, the percentage of cells with seven bivalents ranged from 100 down to 78 (Riley 1959d). Among the long chromosomes of ten interspecific hybrids there were four pairs in 726 cells and three pairs and two univalents in only nine. When the short chromosomes were considered, 690 cells had three pairs, 22 cells had two pairs and two univalents, five cells had one pair and four univalents, and ten cells had six univalents. Cells with fewer or more than the normal number of chromosomes were not included. In both the species and the hybrids there was generally a greater failure of metaphase pairing among the short than among the long chromosomes, possibly because there was less length for the formation of chiasmata. Also, when some chromosomes were missing or there were extra chromosomes, it was usually the short chromosomes that were concerned.

Pairing has been studied in a triploid *Gasteria* and a hypertriploid *Haworthia*. In a *G. sulcata* X *G. nigricans* triploid hybrid (Riley 1948a) most of the long chromosomes were arranged in four trivalents; the next largest group were in three trivalents, one bivalent, and one univalent; a few cells had two or one trivalent. Among the short chromosomes, only one cell of 125 had as many as two trivalents, 11 had one trivalent, and the remainder had only bivalents and univalents or all univalents. Thus it is again seen that synapsis or chiasma formation or both are greater in the long than in the short chromosomes. In two

TABLE 10.1 Chiasma Frequency per Bivalent of Chromosomes of *Gasteria* Plants

Species or Hybrid	Long	Short
G. acinacifolia	1.95	1.0
G. angustifolia	1.95	1.0
G. brevifolia	1.775	1.0
G. carinata	1.925	1.0
G. conspurcata	1.375	0.866
G. dicta	1.625	1.0
G. fuscopunctata	1.625	0.966
G. glabra	1.45	1.0
G. laetipunctata	1.3	1.0
G. maculata	1.525	1.033
G. nigricans	1.8	1.0
G. nitida	1.33	0.966
G. obtusifolia	1.3	1.0
G. parvifolia	1.725	1.0
G. planifolia	1.675	1.0
G. pseudonigricans	1.9	1.0
G. retata	1.675	1.0
G. sulcata	2.0	1.0
G. trigona	1.9	1.0
G. verrucosa	1.425	1.0
G. verrucosa var. *asperrima*	1.425	0.9
G. zeyheri	2.025	1.266
G. X*cheilophylla*	1.8	0.966
G. X*margaritifera*	1.775	1.0

cells four of the long chromosomes were arranged in a quadrivalent. This configuration is interesting since it suggests there are homologous segments in nonhomologous chromosomes. In the *Haworthia* hypertriploid there were three members each of chromosomes L_1 and L_3 and the three short chromosomes, and four each of the L_2 and L_4 (Riley and Majumdar 1966b). Because of these last two chromosomes there were 37 cells out of 70 that had four long chromosomes arranged in quadrivalents. Twenty-nine had trivalents, including 13 that had a quadrivalent, and the remainder of the long chromosomes were arranged in bivalents and univalents. As in plants previously mentioned, pairing was much less frequent in the short chromosomes. Although there were nine short chromosomes, only seven cells of 70 had a trivalent, and nearly all the chromosomes were in bivalents and univalents. There was some indication of homology between normally nonhomologous chromosomes; nine cells had one quadrivalent, four had a hexavalent, and 28 had four bivalents and one univalent, indicating that two apparently nonhomologous chromosomes paired with each other.

Studies of chiasma frequency at metaphase have been few, but the senior author has obtained some hitherto unpublished data for the long and short chromosomes of species and hybrids of *Gasteria*. The results, listed in Table 10.1 show that in the long chromosomes the chiasma frequency per bivalent varies from 1.3 to 2, and for the short chromosomes, from 0.866 to 1.266.

In a plant misidentified as *Gasteria undulata*, which

is not listed in any edition of Jacobsen (1954, 1960, 1970), Darlington and Kefallinou (1957) found that meiosis at first metaphase showed perfectly regular pairing. The long chromosomes had 2.53 chiasmata per bivalent and the short one 1.90. These figures are higher than the present senior author found but reflect the condition in one species only, whereas the senior author found considerable variation among several species. In this plant Darlington and Kefallinou found that the chiasma frequency of the short bivalents was only slightly lower than that of the long ones. The present senior writer found the same general result but a greater difference.

Self-incompatibility

An interesting observation made frequently on plants of the Aloineae is that most plants fail to set seeds under greenhouse conditions. The situation is one of self-incompatibility or self-sterility, to use the older term. Because of their genetic nature, certain plants cannot produce seeds when self-pollinated but frequently produce seeds in abundance when pollinated by other plants of the same species.

The explanation of the genetic basis of self-incompatibility in most plants was given by East and Mangelsdorf in 1925 on the basis of their studies in *Nicotiana*. Their theory proposed a series of "oppositional factors" that can be designated s^1, s^2, s^3, etc., which operate in nearly all plants. In *Nicotiana* an s^1 allele in a female would fail to stimulate the growth of the pollen tube from an s^1 pollen grain. In a self-pollination of an s^1s^2 plant no pollen tubes would grow fast enough to reach the ovules before the flower withered, but in a cross s^1s^2 (female) x s^1s^3 (male) the s^3 pollen tubes are not inhibited and grow at an accelerated rate so that they arrive at the ovary in time to fertilize both s^1 and s^2 eggs and thus produce an abundance of seeds. While this mechanism is the usual one in the plant kingdom, some plants have different genetic (Riley 1936) or cytological (Sears 1937) behavior. In practically all the work in this field in the 1920s and 1930s the phenomenon was termed "self-sterility and cross-fertility"; but the term "self-incompatibility," which was originally proposed by A. B. Stout of the New York Botanical Garden, seems to have prevailed. (East and Mangelsdorf used subscripts, as s_1, s_2, etc., to designate their oppositional factor alleles. To bring these symbols into line with the *Drosophila* work, Riley [1932] converted them to superscripts and they are so indicated in this book. De Haan [1932], in an early extensive review of the gene symbols used by various investigators up to that time, summarized the nomenclatorial systems of many geneticists, some of which were very complicated and most of which differed greatly from one another. He pointed out that Little's committee on genetic form and nomenclature recommended that only exponents be used for a series of multiple alleles and that these superscripts be either letters or numerals; he further commented on Riley's suggested change for alleles at the *s* locus.)

Probably the first reference to self-incompatibility

in the Aloineae was by Berger in 1908. He reported that *Aloe aethiopica, A. pluridens, A. caesia,* and other species failed to set seed after self-pollination although they are very fertile, as are their hybrids. A more extensive observation was reported by Marshak (1934), who found that some species of *Gasteria* (*G. brevifolia, G. nigricans, G. planifolia, G. pulchra,* and *G. verrucosa*) were self-incompatible (self-sterile) while others (*G. lingua, G. disticha,* and *G. sulcata*) were self-compatible (self-fertile). All the species of both classes were cross-compatible (interfertile), so all obviously produced good male and female gametes. When crosses were made, the resulting seed pods were large and contained 45-50 seeds, whereas self-pollinations in the self-incompatible species produced none. When the three "self-fertile" species were self-pollinated, only small seed pods resulted, and they contained only about a dozen seeds. Marshak himself questioned whether these species were really self-fertile or whether they were showing some sort of pseudo-fertility such as East and Yarnell (1929) had reported. This possibility, however, was discounted by Sears (1937). *Haworthia* also is self-incompatible; numerous self-pollinations on four species yielded no seed at all (Majumdar and Riley 1967). Marshak made 19 reciprocal pollinations between four species of *Haworthia* and two species of *Gasteria* and obtained no seed, but factors other than self-sterility alleles may be operating in these intergeneric crosses.

Sears (1937) studied one plant of the self-incompatible *Gasteria verrucosa* (Mill.) Haw. var. *intermedia* Hort., an unidentified self-incompatible species of *Gasteria,* and the self-compatible *G. lingua* (Thunb.) Bgr. Scores of self-pollinations were made on *G. verrucosa* var. *intermedia* during four successive flowering periods, and not one seed was obtained. To compare *Gasteria* with *Nicotiana* cytologically, Sears made many studies of pollen tubes in the style and many paraffin sections for studies of fertilization and ovule development. In *Nicotiana* compatible pollen tubes grew at an accelerated rate whereas incompatible pollen tubes grew at a uniform rate, and too slowly to affect fertilization. In *Gasteria* the percentage of germinating pollen grains was as high after incompatible as after compatible matings — close to 100 percent — showing there is no pollen inviability involved. Furthermore, in contrast to *Nicotiana,* compatible and incompatible pollen tubes grew at the same rate, both entered the embryo sacs, and there was fusion of male and female gamete nuclei. The difference in result between compatible matings on the one hand and incompatible matings or unpollinated flowers on the other hand seems to lie in the development of the integuments of the ovules. An incompatible pollen tube somehow fails to stimulate the integuments to develop normally, apparently as a result of an immune type of reaction between the pollen tube and the integuments. They therefore degenerate, and this degeneration occurs before the endosperm has gone beyond the two-nucleate stage and results in an aborting of the ovule. Furthermore, incompatibly fertilized ovules are not influenced by the presence of compatibly fertilized ovules in the same ovary.

In a study of self-incompatibility in *Gasteria,* Brewbaker and Gorrez (1967) crossed reciprocally plants of two

self-incompatible species, *G. verrucosa* and *G. picta*. (The latter species is designated *G. picta* Bailey but no such species is included in Jacobsen [1954, 1960, 1970]; perhaps it is *G. picta* Haw. which is so included.) By hand they made 3,100 self-pollinations on the two species and obtained no seed pods. Twenty F_1 plants were self-incompatible. Crosses among eleven of them showed that they belonged to four intrasterile, interfertile classes with three, four, two, and two plants, respectively. All groups were fertile reciprocally when crossed with the parents. Brewbaker and Gorrez consider that incompatibility is the result of alleles at one locus acting gametophytically.

The present writers have observed for years that plants of the Aloineae growing in the greenhouse only very rarely set seeds. Such self-incompatibility possibly complicates crossing to some small extent, but attempts at self-fertilization by hand pollination also have been fruitless in many species of *Haworthia* (Majumdar and Riley 1967); even interspecific crosses are usually much more successful than self-pollinations. Resende (1943) believed he had evidence that conditions of the environment could eliminate self-incompatibility in *Gasteria* and *Haworthia* and could also influence the results of cross-pollination. Viveiros (1959) elaborated on Resende's ideas, both from his own observations and from correspondence with Resende, and showed that the environmental factor was insect life. He called attention to the fact that the large number of species and varieties of the Aloineae maintained in the greenhouse of the Lisbon Botanical Garden flower profusely each year but not a single developed capsule has ever been observed. However, when the plants are brought into the open air, large numbers of capsules develop. Berger had stated that insects and other animals bring about pollination in the Aloineae, and the interesting results at Lisbon must be the result of cross-pollination of self-incompatible plants by insects out of doors. Obviously the lack of insect vectors is also the cause of the paucity of enlarged capsules in the writers' greenhouse-grown plants.

Brandham (1969a) corroborated from unpublished data the earlier statements that most species of the Aloineae are self-incompatible. He stated that propagation by seed in the greenhouse would therefore produce hybrids after artificial pollination, since in most greenhouses only one specimen of a species is maintained.

Fertility

Sometimes confused with self-incompatibility is the true sterility or gamete lethality of eggs and sperm. Male fertility is usually determined from the percentage of large, full, deeply staining pollen grains; female fertility from the frequency of large, well-developed fruits with an abundance of seeds. Both methods have their limitations. To determine pollen viability, pollen grains are usually dusted on a slide and a drop of acetocarmine is placed over them. In the writers' studies four replications are usually made for each plant, and each replica-

tion is on a different slide. Ten fields per slide are examined under low power. This method usually reveals both large, swollen, full, red grains and small, shriveled, unstained grains. The latter type apparently comprises only the spore or pollen grain walls and clearly represents sterile and nonviable grains. The large red grains are very different and are considered to be fertile and viable, and the percentage of them is considered to represent the degree of male fertility of the plant. However, there is no assurance that these grains are fertile just because they appear to be, and the assumption that they are may have resulted in erroneous conclusions. To test this possibility, pollen grains were allowed to germinate in 10-15% sucrose with 0.01% boric acid (following the method of Majumdar [1964b]). About 70-80% of the apparently healthy grains germinated, but none of the shrunken, shriveled ones put out a pollen tube.

The determination of egg lethality from the percentage of large capsules with seeds in contrast to completely undeveloped pistils which degenerate and wither soon after they have formed rests on an even more precarious basis. If a plant is egg lethal, its ovaries will not develop and will remain small and shrunken. The differences between developed and undeveloped pistils is perfectly clear, but the cause of the failure of pistils to develop is not. It could easily be the result of egg lethality, but it could equally easily be the result of a failure of pollination and could just as well be caused by self-incompatibility. A pistil that fails to mature because it does not receive compatible pollen is exactly the same in appearance as one with inviable eggs. Unfortunately this result is not appreciated by all cytologists of the Aloineae, who sometimes consider a plant as female sterile when it is really self-incompatible. This failure to understand self-incompatibility sometimes gives an erroneous impression of sterility.

In most genera of plants triploids are highly sterile. Resende and Viveiros (1948) made a cytological survey of a collection of *Haworthia* seedlings, which had been sent to them as *H. coarctata*. On the basis of phenotypic differences the plants were separated into three groups; subsequent cytological examination showed that one group consisted of diploids, a second group of triploids, and the third of pentaploids. The triploids apparently were hybrids between *H. limifolia* var. *limifolia* and a diploid plant from the Coarctatae section. They were highly sterile and produced not a single seed when both self-pollinated and out-crossed to other species. Resende and Viveiros considered these plants as a "fixed hybrid, reproducible only by vegetative propagation."

In a triploid *Gasteria* the senior author (Riley 1948a) found an unusually high percentage of pollen grains that appeared to be viable and fertile. This plant was received from the Huntington Botanical Gardens as *G. sulcata* X *G. nigricans*. The number of pollen grains counted was 750, and of these 46% appeared to be viable. For a triploid plant this percentage is surprisingly high, and the basis for this behavior has not been explained. Even though this percentage is high, it is not as high as that of most diploid species of this genus (Riley 1959d).

TABLE 10.2 Pollen Fertility in *Haworthia*

Species or Hybrid	Chromosome Number	Pollen Fertility (%)	No. of Species or Hybrids
Species	2 *x*	80-100	112
		50-79	24
		0-49	11
	3 *x*	51-100	1
		0-50	9
	4 *x*	80-100	16
		60-79	16
		0-59	5
	5 *x*	80-100	4
		0-79	3
	6 *x*	80-100	4
		0-79	3
	6 *x* - 1 - 1	63	1
	3 *x* + 1 + 1	18	2
Interspecific hybrids	2 *x*	60-100	2
		0-59	2
	4 *x*	71	1
Intergeneric hybrids	2 *x*	91	1

Twelve diploid species had values of 75-98, and only a plant of *G. parvifolia* had below 50% fertile pollen. Seven putative interspecific diploid hybrids varied from 37% to 80%, and four of them had 60% or less.

Majumdar and Riley (1973) have recorded pollen fertility in a number of species or hybrids of *Haworthia* ranging from diploids to hexaploids. The percentages are given in Table 10.2. This table shows that generally among the plants regarded as species the diploids and tetraploids are rather highly fertile although the tetraploids are slightly less so. The triploids, as expected, are largely sterile, and the pentaploids and hexaploids show wide variation. One hypohexaploid plant is moderately fertile and one hypertriploid plant is highly sterile. Interspecific hybrids show a considerable range of fertility and the only intergeneric hybrid is highly fertile. Majumdar (1965) showed there was a high percentage of sterility in two plants that had a heteromorphic bivalent among their long chromosomes.

Sharma and Mallick (1965) made some interesting observations on fertility in the Aloineae in connection with evolution. They pointed out that hybridization occurred between genera "in a number of cases." The present authors also have recorded several intergeneric hybrids. Sharma and Mallick observed that most of the species reproduce mostly vegetatively; they also observed that few seeds or developed capsules are found in plants growing in the greenhouse, which suggests that continued cultivation under horticulture has possibly eliminated their capacity for sexual reproduction. "Meiosis is abnormal for nearly all the species where it could be studied and seed formation is scarce." These statements seem open to criticism. In the first place, meiosis is not abnormal according to

other observations. Taylor (1931), for example, gave a detailed description with excellent illustrations of meiosis in *Gasteria*. From his account of the first and second meiotic divisions and interkinesis, everything seemed perfectly normal. He also gave a careful description of the microspore or first gametophytic mitosis. This division also appeared normal, which it would not if meiosis had been abnormal. While pairing was a little less regular in Kondo and Megata's study of *Gasteria,* and while there seemed to be some precocious separation of one or two of the short chromosomes, these are slight irregularities only and not serious abnormalities. Meiosis also has been regular enough in *Gasteria* as reported by Riley (1959d) and Darlington and Kefallinou (1957). More evidence is needed to support Sharma and Mallick's statement.

One also wonders about Sharma and Mallick's statement that continued cultivation under horticultural practices possibly eliminates the plant's capacity for sexual reproduction. In the first place, there is no *a priori* reason why making continued cuttings would disturb sexual production. In the second place, the problem that interested the authors is apparently speciation. Speciation occurs in the wild and took place long before the advent of greenhouses or horticulturists. Therefore, what would be the importance to speciation of elimination of the capacity for sexual reproduction on the part of plants cultivated in the greenhouse? If such elimination of sexual reproduction does occur in the greenhouse, it affords a good reason for deemphasizing results based on succulent collections and heightens the need for more field studies.

Sharma and Mallick (1965) state that seed formation is scarce and attribute the scarcity, without experimental evidence, to horticultural practices. Obviously the poor seed set they stress is complicated by self-incompatibility if it is not entirely the result of that situation. There is no reason to assume without evidence that the poor seed set results from gamete lethality and to base a theory of the evolution of the Aloineae on that unproved point when the genus is known to be self-incompatible and when self-incompatibility alone could produce the same result.

To try to resolve the difficulty in determining whether the low percentage of seed formation is caused by gamete lethality or self-incompatibility, several self- and cross-pollinations were made by the present authors among species of *Haworthia*. The results are tabulated in Table 10.3. Eleven *Haworthia* species were self-pollinated, and at least ten flowers were used for each plant. One seed pod was enlarged in one plant. The failure of the plants to produce seeds could be explained by pollen or egg sterility; but in view of the studies of Marshak (1934), Sears (1937), Brandham (1969a), and others, self-incompatibility is undoubtedly the explanation. Four crosses were made between varieties, and only two of 62 flowers developed into enlarged fruits. This small percentage of seeds may also be the result of incompatibility genes, because with self-incompatibility alleles — at least of the East and Manglesdorf (1925) "oppositional factor" type — the presence of identical self-incompatibility alleles would result in cross- as well as self-incompatibility.

TABLE 10.3 Fertility in Self- and Cross-Pollinations in *Haworthia*

Species or Hybrid	No. Flowers Pollinated	No. Capsules Enlarged	% Capsules Enlarged
Self-Pollinations			
H. aff. *asperula*	15	0	0
*H. beanii**	10	1	10
H. greenii f. nova	15	0	0
H. limifolia var. *limifolia*	15	0	0
H. limifolia var. *stolonifera* f. *major*	20	0	0
H. longiana (typical plant)	15	0	0
H. reinwardtii var. *olivacea*	15	0	0
H. sampiana [clonotype]	20	0	0
H. semiglabrata	10	0	0
H. skinneri (Bgr.) Res.	10	0	0
H. sp. [received as *H. longifolia*]	10	0	0
Crosses between Varieties of Same Species			
*H. beanii** X *H. beanii** var. *minor*	20	1	5
*H. beanii** var. *minor* X *H. beanii**	12	1	8.3
H. reinwardtii X *H. reinwardtii* var. *olivacea*	15	0	0
H. retusa X *H. retusa* var. aff. *densiflora*	15	0	0
Crosses between Diploid Species			
H. angustifolia var. *albanensis* X *H. limifolia* var. *limifolia*	3	3	100
H. aff. *asperula* X *H. reticulata*	15	0	0
H. aff. *asperula* X *H. retusa* var. *densiflora*	10	0	0
H. aff. *asperula* X *H. schuldtiana*	5	1	20
*H. beanii**(?) X *H. reinwardtii* var. *reinwardtii*	10	2	20
H. longiana X *H. notabilis*	20	2	10
H. longiana X *H. reinwardtii* var.(?)	5	1	20
H. reinwardtii var. *reinwardtii* X *H. beanii**	10	0	0
H. schuldtiana X *H.* aff. *asperula*	5	1	20
H. schuldtiana X *H. longiana*	10	0	0
H. semiglabrata X *H. beanii**	10	4	40
H. semiglabrata X *H. longiana*	10	6	60
H. semiglabrata X *H. reinwardtii* var. *kaffirdriftensis*	10	4	40
H. sp. nov. near *longiana* X *H. jacobseniana* [isotype]	12	8	67
Crosses between Diploid and Polyploid Species			
H. jacobseniana (2*n* = 14) X *H. limifolia* (2*n* = 28)	15	0	0
H. jacobseniana (2*n* = 14) X *H. limifolia* var. *stolonifera* f. *major* (2*n* = 28)	10	3	30
H. longifolia† (2*n* = 14) X *H. sampaiana* [clonotype] (2*n* = 35?)	10	0	0
H. planifolia var. *planifolia* f. *agavoides* (2*n* = 14) X *H. limifolia* (2*n* = 28)	20	2	10
H. retusa (2*n* = 14) X *H. limifolia* var. *stolonifera* f. *major* (2*n* = 28)	10	0	0

TABLE 10.3 (*continued*):

Species or Hybrid	No. Flowers Pollinated	No. Capsules Enlarged	% Capsules Enlarged
Crosses between Polyploid and Diploid Species			
H. limifolia (2*n* = 28) X *H. semiglabrata* (2*n* = 14)	10	1	10
H. limifolia (2*n* = 28) X *H. variegata* (2*n* = 14)	10	1	10
H. limifolia var. *stolonifera* f. *major* (2*n* = 28) X *H. variegata* (2*n* = 14)	12	3	25
H. sampaiana [clonotype] (2*n* = 35?) X *H. longifolia*† (2*n* = 14)	15	0	0

**H. beanii* and its varieties are not listed in Jacobsen (1954, 1970).
†*H. longifolia* is an erroneous designation according to Jacobsen (1954).

Fourteen crosses were made between different diploid species; 32 of 135 flowers pollinated resulted in enlarged capsules. Unfortunately the same plants were not always used for self- and cross-pollinations because often plants did not produce enough flowers. The number of successful pollinations indicates that at least some of the plants were not gamete lethal. *H. semiglabrata* was especially interesting; it produced no enlarged capsules when self-pollinated but produced 14 of 30 in crosses with three other diploid species. Diploid-polyploid crosses are often sterile because of the difference in chromosome numbers, and the development of any enlarged capsules suggests that these plants are not male or female sterile. Taken in conjunction with the results of others, it would appear that the failure of greenhouse plants to set seeds is caused by self-incompatibility rather than by gamete lethality.

Paper Chromatography

Several studies have been made using paper chromatography to identify flavonoids or other chemical compounds, with the idea that an identification of the compounds present in a number of species might lead to a recognition of the relationships of those species to one another. So far these studies have not been extensive. Busch and Resende (1957) made an interesting study of the flower pigments in *Aloe tenuior* Haw. var. *rubriflora* Reyn. and *A. striatula* Haw. var. *caesia* Reyn. The former has red buds and red mature flowers, whereas the latter has yellow buds and flowers. In the F_1 and the heterozygous F_2 plants the buds are red and the mature flowers are yellow, but the F_2 homozygotes are identical with one or the other of the parents. In classic terminology (as Busch and Resende express it) there is a reversal of dominance during the development of the flower, the dominance changing from red to yellow flower color. By paper chromatographic methods Busch and Resende found twelve flavonoids and four carotenoids in the flowers of *A. tenuior* var. *rubriflora*, and

twelve flavonoids, four carotenoids, and one chlorophyll compound in those of the other species. Only two of the flavonoids and two of the carotenoids were common to both species, however. In the heterozygotes they found all the flavonoids of var. *rubriflora,* two of the flavonoids of the yellow parent, and all the carotenoids of both parents, although the concentration of the carotenoids of the yellow parent was low. As the buds open and there is a change from red to yellow, the number of flavonoids of the red-flowered species drops and the carotenoids of that species usually disappear. When the flower is wide open, two or three of the flavonoids of var. *rubriflora* are still present but all the carotenoids of that species have disappeared. In the F_1 also some new flavonoids are present but are ephemeral. Thus there is one new flavonoid in the red bud, two different ones when the bud has become orange, and two others in the yellow open flower. None of these is found beyond its particular stage of floral development. In Lisbon during the winter the heterozygotes cannot produce yellow color and have rose-colored flowers. Temperature apparently affects carotenoid synthesis but not the synthesis of flavonoids. The homozygotes are unaffected.

Three more species of *Aloe* were studied in Lisbon. These species differed from the ones mentioned above in the nature of their pigments and the type of their variation from the bud to the opened flower.

Some years ago the senior author used paper chromatography as a possible means of approaching the questions of taxonomy and biosystematics in the Aloineae. These early studies were concerned with fluorescent compounds and used one-dimensional chromatography. The chemical composition of the spots was not identified. Unfortunately the project was terminated before more complete studies could be made, but the preliminary results were promising and show that further work along this line should be profitable.

A brief mention of the method of paper chromatography might be advisable. Whatman No. 1 chromatographic paper about 55 cm long was used, and a line was drawn near the top along which smears of the mesophyll and epidermis were made. The tissue was pressed into the paper, and spots about 10-15 mm in diameter resulted; these were allowed to dry for about 24 hours. The solvent system consisted of 160 ml n-butanol, 200 ml distilled water, and 40 ml glacial acetic acid — added in that order, shaken for 2 minutes, and allowed to separate for one hour. A chromatographic cabinet (chromatocab) was used, and the lower phase of the above mixture was placed in a tray at the bottom of it. The paper with the leaf tissue was hung in the chromatocab, which was then sealed. An hour later the upper phase of the mixture was added, and the solvent front was allowed to descend until it was about 25 mm from the lower edge of the paper. The chromatocab was then opened and the chromatograms were removed and air-dried for 24 hours. Later they were placed in an oven for 72 hours at 103° C to intensify the fluorescent tracks. Three or four days later they were examined under a black-ray ultraviolet lamp. Spots were observed and were outlined with a pencil, the solvent front was marked, and colors of the various spots were marked on the chromato-

TABLE 10.4 Mean R_f Values and Colors of Spots of Species and Varieties of Coarctatae Section of *Haworthia*

baccata	*coarctatoides*	*fulva*	*greenii* f. *bakeri*	*greenii* f. *pseudocoarctata* Clone 6	*greenii* f. *pseudocoarctata* Clone 12
.092 LB	.099 BB	.111 BB	.112 BB	.112 BB	.112 BB
.209 LB	.181 P	.187 P	.180 P	.199 P	.180 P
.436 LB	.250 BB	.242 BB	.245 P	.238 P	.239 P
.557 LB	.436 MB	.291 LB	.318 MB	.312 MB	.314 MB
	.565 MB	.452 MB	.399 MB	.401 MB	.396 MB
	.641 MB	.574 MB	.483 P	.481 P	.482 P
		.661 MB	.592 MB	.580 MB	.600 MB

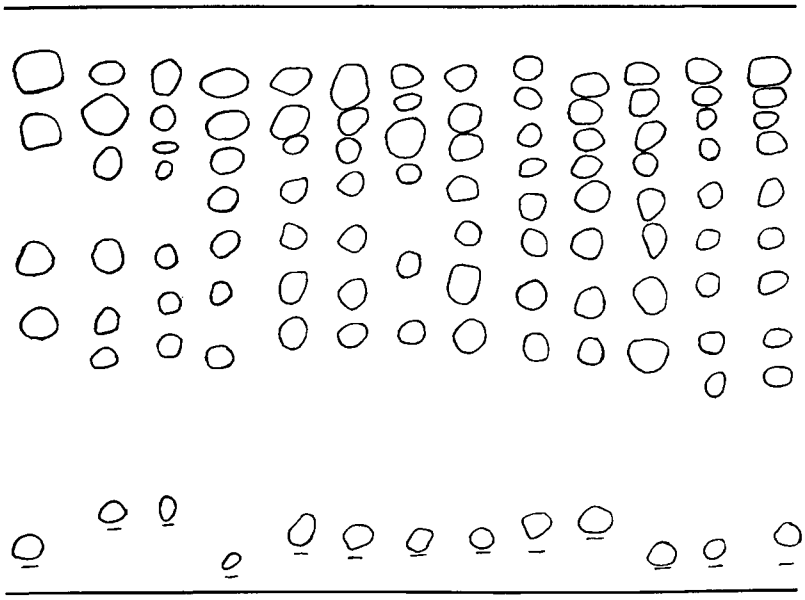
		reinwardtii vars.				
archibaldii	*valida*	*committeesensis*	*tenuis*	*diminuta*	*chalwinii*	*reinwardtii*
.093 BB	.106 BB	.096 BB	.103 BB	.090 BB	.092 BB	.078 BB
.175 DB	.181 P	.143 DB	.157 DB	.140 DB	.139 DB	.116 P
.235 P	.237 MB	.224 P	.223 P	.203 P	.177 P	.154 P
.327 MB	.314 MB	.267 P	.264 P	.255 P	.231 P	.210 P
.481 MB	.391 MB	.346 MB	.348 MB	.311 MB	.303 MB	.284 MB
.593 MB	.487 BB	.416 MB	.427 MB	.397 MB	.389 P	.363 P
	.587 MB	.517 BB	.526 BB	.493 BB	.469 MB	.439 MB
		.610 BB	.624 BB	.580 BB	.578 MB	.558 MB
					.655 MB	.654 MB

LB = light blue; MB = medium blue; BB = bright blue; DB = dark blue; P = pink. Chlorophyll spots are not included.

Numerical values represent average of eight smears.

gram. The R_f value of each spot was determined — the ratio of the linear distance from the center of the spot to the starting line to that of the solvent front to the starting line. The patterns of the spots, including the colors and the R_f values, were then compared. For these studies mesophyll alone apparently is not enough but must be accompanied by epidermis (Riley and Hopkins 1964).

When eight leaves from one plant were studied (Riley and Isbell 1963) the patterns were identical. The number of spots and the color of corresponding spots were the same for each leaf, and the R_f values varied only slightly. The method was consistent within a plant; therefore, it must be reliable and the plant must be uniform throughout, at least insofar as the leaf system is concerned. If the chromatographic profile varied from leaf to leaf, either the method might be unreliable or the fluorescent compounds might not be uniformly distributed or both; the consistency obtained here shows that the method is sound. For several plants chromatographic patterns were obtained in the autumn of 1959 and again in the spring of 1960. Comparisons of the two chromatographic profiles were made for *Haworthia fulva, H. reinwardtii* var. *archibaldiae,* and *H. reinwardtii* var. *reinwardtii*. For each of the three species or varieties the biochemical profiles were identical, showing that no change occurred in the fluorescent compounds over the six-month period. It would have been desirable to examine a number of plants of the same species to learn whether the patterns were species specific, but for most of the species only one individual was available. For *H. greenii* f. *pseudocoarctata,* however, speci-



10.1 Chromatograms of leaf smears from species of Coarctata section of *Haworthia*. From left to right: *H. baccata; H. coarctatoides; H. fulva; H. greenii* f. *greenii* (= *H. greenii* f. *bakeri*); *H. greenii* f. *pseudocoarctata* Clone 6; *H. greenii* f. *pseudocoarctata* Clone 12; and *H. reinwardtii* vars. *archibaldii, valida, committeesensis, tenuis, diminuta, chalwinii,* and *reinwardtii*. (*J.S.Afr.Bot.*, vol. 29)

mens of two different clones could be studied. The chromatographic patterns were identical for four leaves of each of the two clones. This study shows that the patterns are consistent for various leaves of a given plant for at least short periods of time, and probably for different individuals of the same species.

A number of plants of the Coarctatae section have been studied (Table 10.4 and Fig. 10.1). The profiles for seven varieties of *H. reinwardtii* are much alike: vars. *chalwinii* and *reinwardtii* are identical; vars. *committeesensis, tenuis,* and *diminuta* are alike and differ from the first two only by the absence of a pink spot with an R_f value of 0.348-0.393; var. *archibaldii* has six of the spots present in the last three varieties but lacks the blue spot at about 0.513-0.599 and a pink spot at about 0.207-0.248. In var. *valida* six spots are present; it is similar to the three varieties included in the var. *committeesensis* group except that it lacks a dark blue spot and a pink spot near 0.207-0.248 and has an extra blue spot at about 0.240. Three varieties of *H. greenii* are alike and not very different from the varieties of *H. reinwardtii; H. coarctatoides* and *H. fulva* are like *H. greenii* except that they lack two of the pink spots and have an extra blue one. *H. baccata* is different, having only four spots. In general the members of the Coarctatae section that have been studied are very similar.

Fourteen species and varieties of the Retusae section are shown in Figure 10.2. Three varieties of *H. retusa* have profiles similar to one another except that one has an extra spot. In nearly all the species of this section that have been studied, four (sometimes three or five) spots are bunched together near the starting line with one or more spots distributed irregularly toward the solvent front. The pink spots generally prominent in the Coarctatae section are absent from the Retusae section except

TABLE 10.5 Mean R_f Values and Colors of Spots of Species and Varieties of Retusae Section of *Haworthia*

sublimpidula	*mundula*	*retusa*	*retusa* var. *mundula*	*retusa* var. *densiflora*
.135 MB	.106 BB	.090 BB	.103 BB	.152 LB
.220 MB	.180 BB	.132 LB	.152 LB	.202 LB
.258 MB	.210 P	.203 MB	.196 BB	.248 BB
.960 R	.973 R	.321 MB	.278 MB	.382 MB
		.498 LB	.959 R	.974 R
		.954 R		

notabilis	*picta*	*asperula*	*mirabilis*	*maraisii*
.069 MB	.124 LB	.060 BB	.125 BB	.158 LB
.114 LB	.195 MB	.253 BB	.198 LB	.205 LB
.190 DB	.270 MB	.333 LB	.236 LB	.248 LB
.243 MB	.326 DB	.408 MB	.333 MB	.290 LB
.469 DB	.386 DB	.471 MB	.458 MB	.371 MB
.954 R	.954 R	.559 LB	.538 LB	.482 MB
		.658 MB	.689 LB	.569 LB
		.935 R	.962 R	.704 DB
				.769 MB & P
				.954 R

Colors are designated as in Table 10.4, but this table includes chlorophyll spots which are red (R).

Numerical values represent average of four smears.

that one spot is present among those near the beginning line in *H. mundula* and one nearer the solvent front in *H.* sp. aff. *sublimpidula*. (See Table 10.5.)

This method has promise. In general the profiles of varieties of a species are strongly similar and those of different species less so, and those of the Coarctatae section are different from those of the Retusae section.

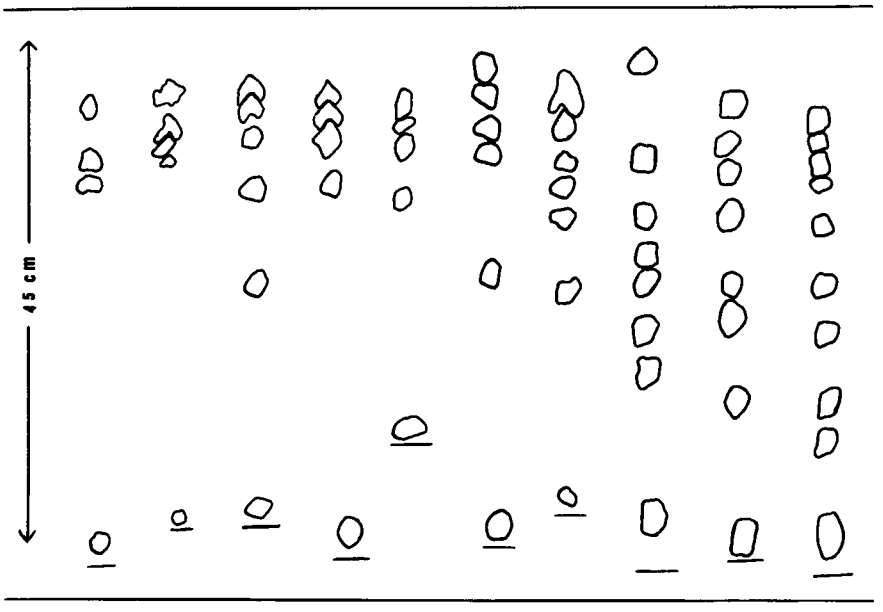
An interesting application of paper chromatography has been made by Brinckmann (1960) in a study of a mutant form of *Aloe lisbonensis*. The normal form has buds that are green when 5-12 mm long, but corresponding buds in the mutant are brown-red. Brinckmann showed that the red color that appears early in the flower buds of the mutant comes from the early synthesis of rhodoxanthin, a carotenoid.

Electron Microscopy

The electron microscope has been used recently by several cytogeneticists to try to obtain further information that would be useful in biosystematic studies of the Aloineae.

Newton (1972) found quite distinctive patterns in the surface relief of the waxy cuticle of the leaves of the West African aloes he studied and later learned that Cutler (1969) had made a similar discovery. Cutler suggested these cuticular patterns might have taxonomic significance but unfortunately he had only one individual of each species.

Newton studied twelve specimens of *A. buettneri* Bgr. from twelve different locations in Ghana, northern Nigeria, and northern Dahomey. He also had one specimen of *A.*

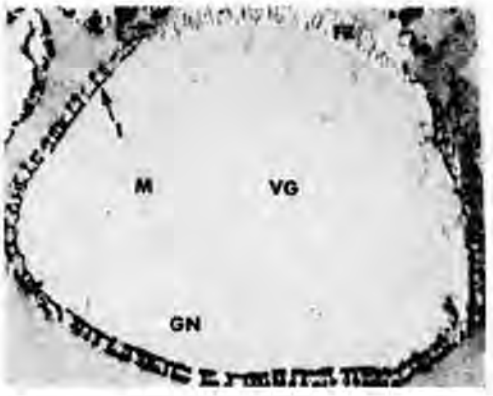


10.2 Chromatograms of leaf smears from species of Retusae section of *Haworthia*. From left to right: *H.* sp. aff. *sublimpidula; H. mundula; H. retusa; H. retusa* var. aff. *mundula; H. retusa* var. aff. *densiflora; H. notabilis; H. picta; H.* aff. *asperula; H. mirabilis; H. maraisii*. (*Taxonomic Biochemistry and Serology*, ed. Charles A. Leone, copyright 1964, The Ronald Press)

keayi Reyn. from the type locality in southern Ghana, and two specimens of *A. macrocarpa* Todaro var. *major* Bgr., one from Ghana and one from Nigeria. He had eight specimens of *A. schweinfurthii* Bak. from Ghana, Dahomey, Nigeria, and Mali. He studied the abaxial surface of the leaves and used both a light microscope and a scanning electron microscope.

The surface of the cuticle was complex, consisting of ridges and papillae, with the ridges sometimes anastomosing to form a reticulate pattern. The stomata were sunken, and prominent craterlike structures formed the entrances to the stomatal pits. In Newton's material two taxa had symmetrical apertures, and in two other taxa the sides of the apertures were folded inward. In *A. buettneri* there was a reticulate pattern of ridges on the surface and the stomatal apertures were of the symmetrical type. In *A. schweinfurthii* the surface was covered with individual papillae, and each corresponded to an epidermal cell below it. This basic pattern varied in a few plants; in some a few of the papillae extended into short ridges, and in one clone some low ridges radiated from the papillae. The stomatal apertures were of the symmetrical type. In the only plant available of *A. keayi* a papilla was often present in a cell area and was surrounded by a rosettelike arrangement of short ridges. The stomata were of the asymmetrical type. In *A. macrocarpa* var. *major* the pattern was one of reticulate ridges and the stomata were of the asymmetrical type.

Although there was some small intraspecific variation, generally a pattern could be recognized for the two species for which an adequate number of clones was present for study. The method seems to offer considerable promise for taxonomy. Furthermore, it can possibly be used to study the species of all herbarium specimens, either to verify the determination of the original collector or to



10.3 Electron micrograph of pollen wall of *Gasteria conspurcata* showing a germinal pore. FR: electron dense channels; VG: vegetative nucleus; GN: generative nucleus; I: intine; E: membrane over the pore. (*Grana*, vol. 11)

identify a specimen the original collector could not identify.

Other investigators (Majumdar and Lowry 1971a) have used the electron microscope for ultrastructure studies of the pollen grains of *Gasteria armstrongii*. Their study revealed the existence of a barrier between the vegetative and generative nuclei. The barrier had a middle lighter zone bordered by two dense lines. The layer was continuous with the intine of the pollen wall.

Ultrastructure studies of the pollen wall morphology of the Aloineae have been employed (Majumdar and Lowry 1971b; Majumdar 1972) to assess the interspecific and intergeneric relationships, especially as they relate to taxonomy and biosystematics. One report dealt with the electron microscope study of the *Gasteria* pollen wall (Majumdar and Lowry 1971b), which showed an identical pollen wall anatomy of two species (*G. armstrongii* and *G. conspurcata*) (Fig. 10.3), a hybrid (*G.* X*cheilophylla*), and an unidentified plant designated *G. regina*. The exine of the pollen wall was made up of ektexine but no endexine. The ektexine was composed of a tectum forming the outer layer, columellae which supported the tectum, and an inner foot layer. There was one germinal pore in the pollen grain. The tectum, columellae, and foot layer stopped abruptly around the rim of the vestibulum, and the pore aperture was formed. The intine was a thin layer under the nonapertural exine which thickens in the aperture.

Recently (Majumdar 1972) fine structure of the pollen wall stratification of *Astroloba spiralis, As. skinneri, Haworthia viscosa,* and *H. limifolia* was studied to determine the taxonomic relationship among the species and genera. As before, the ultrastructural pollen morphology of the species of the two genera showed the presence of the ektexine but not the endexine. The tripartite ektexine was composed of the tectum, columellae, and the foot layer; and the pollen wall stratifications of the interspecific plants showed no difference in morphological makeup (Fig. 10.4). However, this study showed some differences of the pollen wall anatomy between the two genera. *Haworthia* ektexine exhibited a thick foot layer with a discontinuous tectum. The pollen wall of *Astroloba,* on the other hand, was found to have a much thinner foot layer. A dark layer was found to traverse the columellae and characteristically to form a thick layer over the tectum.

More recently Cutler and Brandham (1977) published a study on leaf surface characters in hybrids that promises to be only the first of a series of interesting papers on this problem. This subject is an important one because leaf characters are essentially unchanged by the methods used to prepare herbarium specimens and, if they are shown to be specific for the various species and varieties, can be used to verify the identification of plants that were collected, identified, and studied many years ago. Cutler and Brandham's studies were based on the eight bigeneric hybrids listed at the end of Chapter 11. Seven were between *Gasteria* and *Aloe* and one was between *Aloe* and *Haworthia*. Several easily recognizable anatomical characters were considered and will be described here briefly.

The epidermal cells that are not associated with tubercles are mostly 5- or 6-sided, but in *G. lutzii* are 4-

to 7-sided. They may be small or may be variable in size. Sometimes they are slightly longer than wide but they may be up to twice as long as wide in some species. The outer periclinal wall, as viewed from the surface, may or may not have patterns such as one formed from numerous small lumps or micropapillae which sometimes fuse to form ridges or reticulate patterns. The micropapillae are sometimes obtuse and small and may form a conspicuous pattern on the outer wall.

Larger prominent outgrowths from the outer walls are often found. They are the papillae and are different from the micropapillae. They may be hemispherical or conical and prominent but may be absent, as in *G. carinata* X *A. dorotheae*. The stomata are usually sunken, often deeply so, but are sometimes only moderately or slightly sunken; in some plants, as in *A. tenuior* var. *rubriflora*, they are superficial. They are usually surrounded by four lobes which may be upright or overarched and are often of more or less equal length. They may be tall and upright and of an even, moderate thickness. The aperture which is formed by the surrounding lobes may be square or elongated and rectangular.

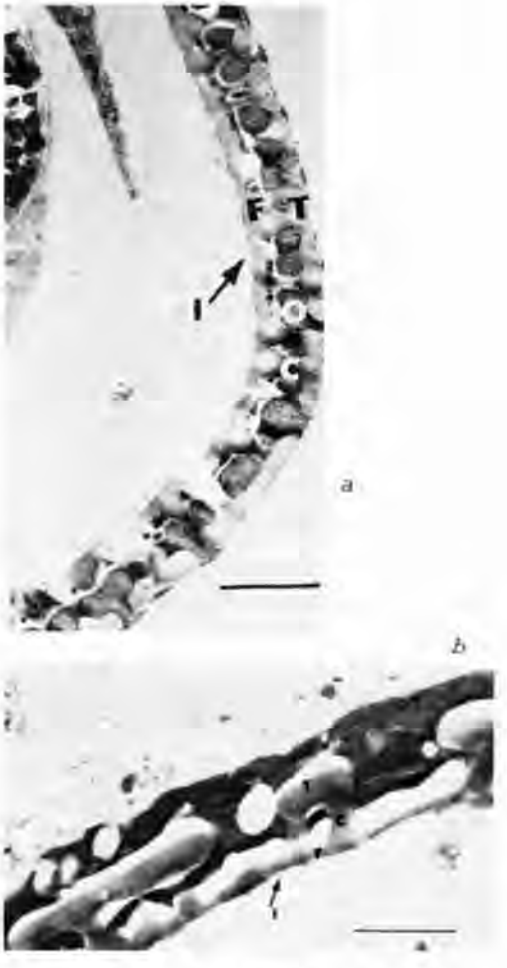
The anticlinal walls may or may not have a pattern. They may be marked by prominent raised bands, slightly raised bands, conspicuous, broad bands, flat-bottomed furrows, parallel bands with grooves between, raised and conspicuously lumpy bands, and other variations. The surface of the leaves is covered with a layer of wax which may vary in nature from species to species. It may be present only as a more or less continuous sheet or as scattered particles which may be small or large and conspicuous or inconspicuous.

These many differences in leaf surface characters afford a wealth of variation for genetic studies. Cutler and Brandham recognize that this study includes too small a sample to permit general conclusions to be drawn, but they feel that some of their observations probably have wider significance for the tribe. A striking feature of this work is that few, if any, characters can be considered dominant. Usually the hybrid is intermediate, as is well shown for the stomata in the hybrid *G. lutzii* X *A. tenuior* var. *rubriflora*. In the first species the stomata are sunken, in the second they are superficial, and in the hybrid they are intermediate. The lobes surrounding the stomata are present in the first species, absent in the second, and but slightly developed in the hybrid.

The inheritance of papillae must be complex. A cross between two plants that lack papillae results in plants without papillae. *G. carinata* has papillae but when crossed with *A. dorotheae*, which lacks papillae, produces plants without papillae; with the slightly papillate *A. antandroi* it produces papillate offspring. *G. planifolia*, which is papillate, crossed with *A. dorotheae*, yields offspring that have poorly developed papillae.

Micropapillae also show essentially the same condition. If a plant with micropapillae is crossed with one that lacks them the F_1 tend to have a poorly developed pattern.

From these studies Cutler and Brandham apparently feel that there is no condition of simple dominance in leaf



10.4 Portions of pollen grain wall of (*a*) *Haworthia viscosa* and (*b*) *Astroloba spiralis*, as seen with the electron microscope. F: foot layer; C: columella; T: tectum; O: osmophilic substance; I: intine. The line equals 1 μm. (*30th Annu. Proc. Electron Microscopy Soc. Am.*)

surface characters in the Aloineae. The intermediate condition is normal but sometimes a character appears in the hybrid that is unlike that in either parent and that can be considered "new." As Cutler and Brandham suggest, it may result from genome interaction. Unpublished data of theirs suggest that there is uniformity within a species and they hope to establish this point in future studies.

Majumdar and his student (Majumdar and Sullender 1977) have studied the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on various cell structures of *Haworthia* callus as seen with the electron microscope. This paper is part of a project showing the effects of several herbicides (2,4-D and 2,4, trichlorophenoxyacetic acid) and other chemical compounds on various cell parts, including chromosomes.

The high concentrations of 2,4-D induced tumor-type growth in the callus tissues. The callus was obtained from the axes of flowers of *Haworthia variegata* and was induced and grown on the medium mentioned in Chapter 9. These tissues are much easier to handle than are mature plants and are presumably just as reliable for indicating the effects on cells of various compounds. The callus on the control medium developed normally (Fig. 10.5) but that on the medium with herbicides had dark pink pigmentation and many very small nodules (Fig. 10.6). In several instances the callus showed tumorlike growth (Fig. 10.7) but little differentiation, and sometimes the roots were fat and stubby.

Under the electron microscope the parenchyma cells of the controls had a narrow band of protoplasm with a definite nucleus, some plastids and mitochondria, a large cen-

a

b

10.5 (*a*) *H. variegata* callus culture after two weeks of inoculation; many budlike nodules are seen; (*b*) seven-week-old culture of *H. variegata* showing plantlets, roots, and callus. (*Am.Biol.Teach.*, vol. 39)

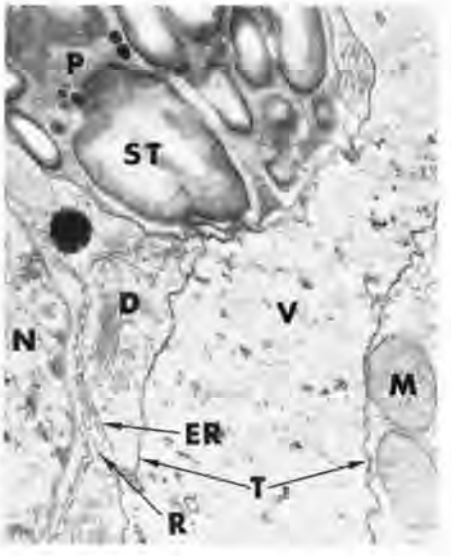
10.6 Production of tumor nodules 10 weeks after inoculation of callus on medium containing 10 mg/l of 2,4-D. (*Phyton*, vol. 35)

10.7 Tumor-type growth induced by 10 mg/l of 2,4,5-T in *H. variegata* callus cells grown *in vitro*. (*Am.Biol. Teach.*, vol. 39)

tral vacuole with a clear tonoplast, some cytoplasmic vacuoles and a few dictyosomes with stacks of cisternae having vesicles at the ends (Fig. 10.8). There were ribosomes, and the endoplasmic reticulum was generally of the smooth type. Plastids frequently possessed osmiophilic granules and starch grains of various sizes. Mitochondria were few and, when present, always had the typical pattern of cristae.

In the tumor cells the nucleus and plastids were no different from those in the normal cells but frequently the endoplasmic reticulum of the cells treated with 2,4-D was somewhat dilated. In the tumor cells there were often a high degree of vacuolation, breaks in the tonoplast, and electron-dense particles on the tonoplast (Figs. 10.9, 10.10). There were also more free ribosomes and polysomes. The mitochondria of the treated cells often had dilated cristae and vesicle-like bodies in the matrices (Figs. 10.11, 10.12).

Chromosome studies on callus tissue have not been so extensive as have studies on the ultrastructure of the cell, but a few such studies have been carried out. Sodium cyclamate brought about changes in the growth patterns of callus cells of *Haworthia* (Majumdar and Lane 1970), and the carcinogen 7,12-dimethylbenz(a)-anthracene stimulated root production of callus (Majumdar and Newton 1972), but neither caused chromosome aberrations of the kind produced by radiation and some chemical compounds. Since these chemical compounds caused chromosome damage in animals, it is interesting to find their inability to induce chromosome abnormalities in plants; these results are in accord with some studies of the effect of lysergic acid diethylamide tartrate (LSD) on chromosomes of *Vicia faba* and *Allium cepa* (Riley and Neuroth 1970).

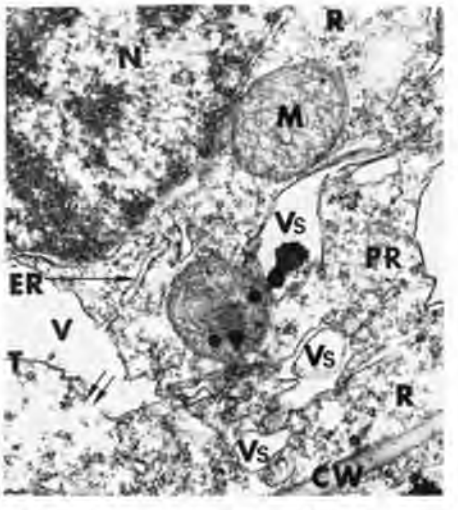


10.8 Portion of a normal cell: note starch (ST) and osmiophilic granules in the plastid (P), mitochondria (M) with normal cristae pattern, smooth endoplasmic reticulum (ER), and the vacuole (V) bordered by intact tonoplast (T). D: dictyosome; N: nucleus; R: ribosomes. (*Phyton*, vol. 35)

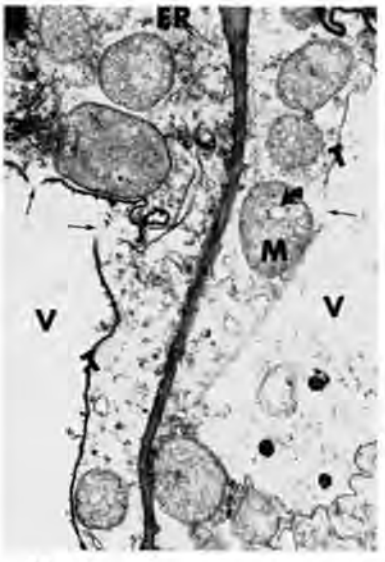
Inheritance of Leaf Pigmentation

Although cytological, and especially chromosomal, studies on the Aloineae have been abundant during the last half-century, genetic studies have generally been few. It has been shown that self-incompatibility (self-sterility) is present in many plants of the tribe and that it is under genetic control. The exact mechanism, however, and the number and kinds of genes involved have not been established as they have in genera such as *Nicotiana*. Studies on fertility and hybridization have provided some information on the genetic background of the Aloineae, but the extensive pedigree culture studies that have revealed the presence of many genes in many other groups of organisms have not been carried out.

A study of leaf pigments in *Gasteria* has recently been made by Brandham (1977c). The typical species of this genus has dark green leaves with many pale green spots distributed over both upper and lower surfaces. In a few species, however, the leaves are pale green all over the surface and no spots are observed. One of these light green species is *G. bicolor*. One plant of this species was available in the Tropical Department of the collection of living plants at Kew and was crossed as the male parent with *G. "amoena"* Hort., *G. Xcheilophylla* Bak., *G. lili-*

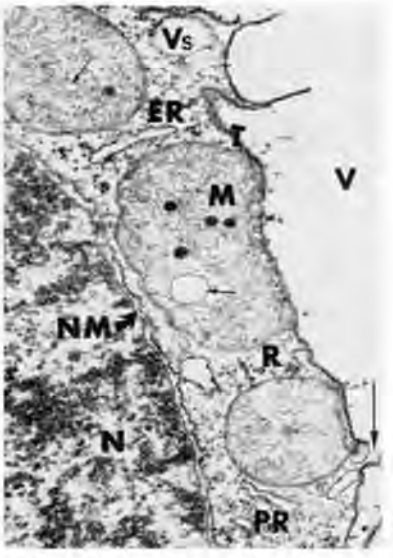


10.9 Tumor cell (20 mg/l). N: nucleus; R: ribosomes; PR: polyribosome; Vs: cytoplasmic vacuoles; T: tonoplast; ER: endoplasmic reticulum; CW: cell wall; V: vacuole. Note dilated ER (*arrow*) and broken tonoplast (*double arrow*). (*Phyton*, vol. 35)



10.10

10.11



10.12

10.10 Tumor cells (10 mg/1): note the breaks in the tonoplast (*arrows*) and accumulation of electron dense particles on tonoplast (T); a mitochondrion (M) with a vesicle-like body (*thick arrow*) can be seen. V: vacuole; ER: endoplasmic reticulum. (*Phyton*, vol. 35)

10.11 Vesicular bodies (*arrows*) in the mitochondria of a tumor cell (20 mg/1) are visible; note two dictyosomes (D) and several cytoplasmic vacuoles (Vs). (*Phyton*, vol. 35)

10.12 A tumor cell (10 mg/1) showing a nucleus (N) and several mitochondria (m); vesicles in the mitochondria (*arrow* in the mitochondrion), deposition of electron dense particles on tonoplast (T), and broken tonoplast (*arrow*) are evident. Vs: cytoplasmic vacuole; ER: endoplasmic reticulum (note the dilation); R: ribosomes; PR: polyribosomes; NM: nuclear membrane; V: vacuole. (*Phyton*, vol. 35)

putana v. P., *G. parviflora* Bak., *G. planifolia* Bak., *G. poellnitziana* Jacobs, *G. subverrucosa* (Salm-Dyck) Haw., and *G. verrucosa* (Mill.) Duv.; and, as the female parent, with *G. spiralis* Bak. The F_1 plants from these nine crosses segregated into 74 self-color pale green and 71 green spotted, a ratio that approaches 1:1 very closely. Fifteen crosses were made among F_1 plants to test further generations; they all involved crosses from the *G. biloba*, *G. liliputana*, and *G. verrucosa* original plants. When the F_1 plants crossed were a spotted and a green (there were ten such crosses), the offspring segregated into 242 green and 232 spotted. When three combinations were selected where both parents were green, the offspring segregated into a clear 3:1 ratio (134 green and 49 spotted). Two crosses of spotted x spotted gave 171 spotted and no all-green plants. As Brandham showed, the difference between green and spotted plants is the result of a single gene with the green allele dominant over the spotted. The original green plant must have been heterozygous.

Crosses involving *G. humilis* as the male parent and *G. liliputana* as the female produced several disturbed ratios in the F_1 and F_2 generations that were very interesting and suggested that several different genes were involved. Some of the F_2 seeds were nonviable and in some crosses albino seedlings appeared in from 13 to 48% of the progeny, apparently the result of several harmful alleles that are apparently recessive. The number of such recessive genes has not yet been learned but at least two must be present, because in some crosses of F_1 plants ratios were found approximating the 9:7 ratio that Bateson *et al.* discovered in the sweet pea in 1905. Genes that result in this ratio bear Bateson's classical term "complementary factors." In these *Gasteria* crosses, two pairs of alleles interact. In each pair normal is dominant over albino but when two pairs of "complementary" genes interact, the normal phenotype occurs only when the dominant allele of both pairs is present.

Chapter Eleven

HYBRIDIZATION

The possibility that natural hybridization occurs in the Aloineae has been suggested for almost a century. In Berger's article in *Das Pflanzenreich* (1908) hybridization is discussed and a large number of putative hybrids are listed. They are supposedly hybrids that arose in nature, since the creation of hybrids by artificial pollination was not extensive then. The decision that a plant was or was not a hybrid was based on an analysis of its phenotypic characters and a comparison of them with those of other plants of the same genus. This method of comparative morphology demands a detailed understanding of all plants of the same and related genera and is susceptible to errors.

In *Aloe,* Berger listed about 35 probable hybrids. Some were simply given a specific name and a hybrid designation, as *A.* X*prorumbens* Baker. Some had descriptive terms, as *A.* X*bortiana* Terrac. f. ("Garten-hybride"), *A. spuria* Bgr. n. sp. ("vielleicht nur hybriden Ursprungs"), and *A.* X*schimperi* Tod. ("Nil nisi hybrida *A. striatae* ut frequenter ex ejus seminibus in hortis nascitur"). After some apparent hybrids the putative species were mentioned, as with *A. arborescens* Mill. var. *ucriae* (Terrac. f.) Bgr., where it was written, "Sed mea opinione vix species propria, potius hybrida inter *A. arborescentem* et *A. pluridentem,* inter quas fere medium tenet." After *A.* X*spinosissima* Hort., the putative parents were given as, "Forsan hybrida *Aloë humilis* var. *echinata* X *A. arborescens* var. *pachythyrsa.*"

A dozen or so hybrids were given for *Gasteria,* but the probability of many more was suggested. The author sometimes mentioned that there are many garden varieties and hybrids but only a few are worthy of being listed. For some species, as *G. brevifolia,* there were vague statements: "In hortis hybridae variae occurrent." For others, as *G. conspurcata* (S.D.) Haw., references were more specific; here, referring to a figure of Salm-Dyck showing a juvenile plant, the author wrote, "Medium tenet inter *G. angustifolium* et *G. angulatam.*"

Only six *Haworthia* hybrids were listed by Berger (1908). They included *H. fasciata* var. *caespitosa* Bgr., which was "perhaps" a hybrid between *H. fasciata* and *H. attenuata; H. semiglabrata,* designated as perhaps a hybrid; and *H. subattenuata,* probably a hybrid form. *H. recurva* was thought by Salm-Dyck to be a hybrid of *H. venosa,* and the same author considered *H. viscosa* var. *indu-*

rata (Haw.) Bak. to be a hybrid between *H. viscosa* and *H. cordifolia*. *H. hybrida* (S.D.) (Haw.) also was classed as a hybrid, but its putative parents are in dispute. Baker believed they were *H. rigida* and *H. radula*, whereas Berger considered them *H. rigida* and *H. tortuosa* and Salm-Dyck thought they were *H. margaritifera* and *H. tortuosa* var. *pseudorigida*.

Apparently no plants of *Astroloba* (*Apicra*), *Chamaealoe*, *Chortolirion*, or *Lomatophyllum* are hybrids according to Berger. These are small genera and would not be so likely to produce many hybrids. *Chamaealoe* is monotypic, *Lomatophyllum* has three species, *Chortolirion* has four, and *Astroloba* (the largest) has only nine, as listed by Berger. In *Gasteria* a number of intergeneric hybrids are included. One is *G. holtzei* (= *Gasterhaworthia* X*holtzei*), which is supposed to be a hybrid between *G. verrucosa* var. *intermedia* and *Haworthia radula*. Other hybrids believed to be between these two genera are *G. bayfieldii* (S.D.) Bak., *G. apicroides* Bak., and *G. squarrosa* Bak. All have been cultivated in Kew.

Twelve putative hybrids are mentioned under *Aloe*. *A.* X*beguinii* Hort. is such a hybrid and can be propagated by leaf cuttings, as can apparently all species of the genus *Gasteria*. *A.* X*beguinii* var. *perfectior* Radl., *A.* X*chludowii* Beg., and *A. lynchii* Bak. apparently involve *G. verrucosa* as one parent; *A. lapaixii* Radl., *A.* X*bedinghausii* Radl., and *A. simoniana* Del. have *A. aristata* as the *Aloe* parent. Other designated *Aloe-Gasteria* hybrids are *A.* X*smaragdina* Hort. Bgr., *A.* X*mortolensis* Bgr., *A.* X*derbetzii* Hort. Bgr., and *A.* X*rebutii* Bgr. *Aloe* X*bedinghausii* is often listed under the hybrid genus *Gastrolea* as *G.* X*bedinghausii* (Radl.) E. Walth. Similarly, *A.* X*beguinii* is often listed as *G.* X*beguinii* (Radl.) E. Walth., *A.* X*chludowii* as *G.* X*chludowii* (Beg.) E. Walth., *A.* X*derbetzii* as *G.* X*derbetzii* (Hort. Bgr.) E. Walth., *A.* X*lapaixii* as *G.* X*lapaixii* (Radl.) E. Walth., *A.* X*lynchii* as *G.* X*lynchii* (Bak.) E. Walth., *A.* X*mortolensis* as *G.* X*mortolensis* (Bgr.) E. Walth., *A.* X*rebutii* as *G.* X*rebutii* (Bgr.) E. Walth., *A.* X*simoniana* as *G.* X*simoniana* (Del.) Jacobs, and *A.* X*smaragdina* as *G.* X*smaragdina* (Bgr.) E. Walth. *Aloe* X*beguinii* var. *perfectior* has been raised to specific status under *Gastrolea* as *G.* X*perfectior* (Bgr.) E. Walth. *Aloe* X*hoyeri* is frequently listed as *Lomataloe* X*hoyeri* (Radl.) Guill. *A.* X*hoyeri* Radl. apparently arose from a cross of *Lomatophyllum borbonicum* and *Aloe serrulata*. According to Berger no crosses have been carried out between *Aloe* on the one hand and *Chamaealoe*, *Astroloba*, and *Haworthia* on the other. The purpose of reviewing this old work is largely to emphasize that the concept of hybridization in the Aloineae is far from new.

In his two large volumes on the aloes, G. W. Reynolds listed many putative hybrids. In *The Aloes of South Africa* (1950), 174 entirely intrageneric hybrids were included and the two putative parents of each were named. *A. marlothii* Bgr. was considered to be one of the parents of 40 hybrids, *A. arborescens* Mill. was apparently a parent of 25, and *A. ferox* Mill. was believed involved in 17. These species are widely disseminated and therefore could easily find a number of different species with which to mate. In his second volume (*The Aloes of Tropical Africa and Mada-*

gascar, 1966) Reynolds listed only 20 hybrids involving *Aloe* species.

Jacobsen (1970) believes that considerable hybridization has taken place in some of the genera of the Aloineae. In small genera such as *Astroloba, Chamaealoe, Chortolirion,* and *Lomatophyllum* apparently there has been no hybridization within the genus. In *Aloe* Jacobsen lists 32 putative hybrids; one is an intervarietal hybrid, but the others are interspecific. Eight putative hybrids are suggested in *Gasteria,* all of which have two *Gasteria* species as parents; this list does not include a garden form which may have a hybrid origin, perhaps remote. Six putative hybrids are listed in the genus *Haworthia.*

Several intergeneric hybrids are interesting. Eighteen named hybrids and a variety of one of them constitute the genus *Gastrolea* E. Walth., some of which are mentioned above. Three hybrids of *Gasteria* and *Haworthia* are listed under the hybrid genus X*Gasterhaworthia* Guill.; they include the rather well known X*G. bayfieldii* (S.D.) Rowl. and X*G. holtzei* (Radl.) Guill. A hybrid between *Gasteria* and *Chortolirion* has been listed as X*G. orpetii* E. Walth. in the hybrid genus X*Gastrolirion* E. Walth. Two other hybrid genera that Jacobsen (1970) includes are X*Lomataloe* Guill. and X*Lomateria* Guill., each a monotypic genus. X*Lomataloe hoyeri* (Rad.) Guill. is a hybrid between *Lomatophyllum purpureum* and *Aloe serrulata,* and X*Lomateria gloriosa* (Radl.) Guill. is a hybrid between *Lomatophyllum purpureum* and *Gasteria maculata.* The latter at least must be an artificial hybrid because *Gasteria* is a plant of South Africa and *Lomatophyllum* is a genus of Madagascar, Mauritius, and some neighboring islands; the two genera are well separated by geographical barriers.

A number of other writers have studied or written about hybrids. In one of the earliest cytological studies Taylor (1924), who had difficulty as do many others with identifying his material, stated: "Most of the material came from plants fitting best the descriptions of *Gasteria verrucosa* (Mill.) Haw. and *G. Cheilophylla* Baker or intermediates between them." Sato (1937) had some interesting evidence of hybridization based on the heterozygous condition of satellites. By artificial pollination he and Sinoto produced three intergeneric hybrids: *Aloe variegata* X *Gasteria verrucosa* var. *latifolia, A. variegata* X *G. "Gyu-zetu"* (not otherwise identified), and *G. "Gyu-zetu"* X *A. variegata.* They generally showed matroclinous characters, which the author attributes to cytoplasmic influence, but also showed definite evidence that they were intermediate between the two genera. They also indicated very definitely that the plants are not gamete lethal and that failure of seed set cannot always — if ever — be attributed to the failure of the gametes to function, as has been suggested by some cytologists. (See Chapter 10 above.) A detailed analysis of the karyotypes of these plants was published by Sinoto and Sato (1940) and indicates their hybrid nature. Aberrations found in them include several inversions and translocations.

Müller (1945) listed and described two putative *Aloe* hybrids. One was *A. castanea* Bak. X *A. globuligemma* Pole Evans. It was found in the Steelpoort region with *A. globuligemma* and *A. castanea,* and on the basis of growth

form and morphology of the parts of the plants it was considered a hybrid between these two species. The other hybrid was *A. arborescens* Mill. x *A. chortolirioides* Hort. It was found 48 km east of Barberton and, at the time the paper was written, was growing in Dr. Reynolds's garden in Johannesburg; a cutting was also growing in the garden of Dr. Müller and was used to describe the plant. Reynolds stated that this plant was found near the two putative parents. Müller cites a paper by Fernandes (1930) in which the latter mentions several putative hybrids, namely *A.* x*winteri* Bgr., *A.* x*spinosissima* Hort., and *A.* x*paxii* Terrac. f. Of these three none is listed in Reynolds (1950, 1966) and only *A.* x*spinosissima* in Jacobsen (1954, 1970).

Resende and his colleagues considered that much hybridization occurred and cited many *Haworthia* plants as hybrids. Some of them were chromosome number hybrids, and plants with odd-numbered chromosome sets are readily interpreted as hybrids (Resende and Pinto-Lopes 1946). For example, plants with 5*x* chromosomes are easily assumed to be the result of a cross between 4*x* and 6*x* plants. They include *H. sampaiana* Res. (5*x* + 1) and *H. sampaiana* f. *broteriana* Res. and Pinto-Lopes (5*x*); while they are considered to be numerical hybrids, their putative parents are not even suggested. Another probable hybrid is the triploid *H. reinwardtii* var. *archibaldiae,* which is assumed to be a hybrid between varieties *major* and *conspicua*. Being a triploid, it may well be of hybrid origin, but the varietal differences in this species are so small that any attempt to suggest the putative parents is dangerous. *H. cassytha* Baker is a diploid but does not flower; since nearly all *Haworthia* species flower vigorously, Resende and Pinto-Lopes suggest it is a hybrid, probably between *H. lisbonensis* Res. and *H. tortuosa* or a closely related species. The problem with this suggestion is that many hybrids, or at least putative hybrids, produce flowers just as abundantly as do putative species. Therefore, to single out one plant that does not flower and call it a hybrid because it does not flower ignores all the putative hybrids that do flower. Other kinds of evidence are needed.

Resende and Pinto-Lopes further suggest that three forms of *H. greenii* (fs. *greenii, minor,* and *pseudocoarctata*) are hybrids; they hesitate to name the putative parents and consider that two may be Mendelian segregates of the third, indicating that only field studies and breeding studies may answer the question. A triploid plant, *H. resendeana* v. Poell., which flowers abundantly, is possibly a 2*x* x 4*x* hybrid according to Resende and Pinto-Lopes (1946). *H. skinneri* (Bgr.) Res. neither produced flowers nor reproduced vegetatively and is presumed to be a hybrid, but the plant died before it could be studied. It is only mentioned in Jacobsen with the notation that it is not *Astroloba skinneri* (Bgr.) Uitew. Resende and Pinto-Lopes sum up their argument:

> In fact, the plants of the Sub-tribu [*sic*] *Aloineae* very easily give hybrids.... The hybridity alone and the spontaneous mutations, caused by the hybridity ...or not, are certainly a frequent mechanism of the

> origin of new strains with different ranges: species, varieties a.s.o. [*sic*]. Moreover, in the genus *Haworthia* and chiefly in sections *Tessellata* [*sic*] and *Coarctatae,* changes in chromosome number — polyploidy — are frequent.... We are therefore confronted with a group of plants in active evolution.... The genotypic identity or the genotypic differences in the genus *Haworthia* and *Gasteria* can be recognized only when the individuals are grown, side by side, in absolute identity of milieu conditions and during several years.

Pinto-Lopes (1946) added two hybrids to the list of Resende's group: *H. herrei* v. Poell. X *H. reinwardtii* var. *minor* Bak. and *H. jacobseniana* X *H. reinwardtii* var. *minor* Bak., both diploids. Three new strains of *Haworthia* were reported by Resende and Viveiros (1948) with 3*x*, 5*x*, and 6*x* chromosomes. The two odd-numbered polyploids were hybrids from parents of the Margaritiferae and Coarctatae sections. The most significant statement in their paper relates to speciation: "In our opinion, the speciation in the Coarctatae-Section is fundamentally due to hybridism and its genetic consequences...or to the combination of this with polyploidy." Viveiros (1949) confirmed this statement and added, "Surely all this intraspecific genetically determined variability of *Haw. tesselata* [*sic*] is the consequence of the combined hybridism and polyploidy." He especially pointed out the hybrid nature of strains of odd chromosome number and of aneuploids and admitted that some strains of even number might also be of hybrid origin. Resende (1949) emphasized that in the Aloineae intergeneric and interspecific crosses are very easy to make, complicating the problem of taxonomy, but added no new examples in that paper.

Viveiros (1959) made a number of crosses between various species with different chromosome numbers and found that no degree of polyploidy from diploid to hexaploid was a barrier to intercrossing. He emphasized that in the Coarctatae section there were many polyploid forms and that many members were probably hybrids. Pinto-Lopes (1944) had found a natural polyploid series in this group containing apparent hybrids, and Resende and Pinto-Lopes (1946) concluded that some plants of his group had a hybrid origin. Viveiros went even further and concluded that the whole tribe of the Aloineae, not only the Coarctatae section, is "still an evolving group." Resende (1949) stated that hybridization and polyploidy complicate the study of the taxonomy of the group, especially when the taxonomist is strange to the area where the plants are found. Viveiros studied 356 plants obtained from seeds and involving 14 species and varieties. They were grown in the Botanical Garden in Lisbon, and the original plants and the F_1 generation were all grown under the same conditions of environment. For this reason the differences in the morphology of the plants are assumed to be genotypic. In the F_1 there were many differences in chromosome numbers, but no correlation could be established between chromosome number and the morphology of the plants. This diversity of chromosome number led Viveiros to state that most of the F_1 plants arose from interspecific crossing: "This conclusion is not surprising as Berger (1908), Re-

sende (1943) and Resende and Viveiros (1948) have called attention to the great ease with which these plants form hybrids."

A number of putative hybrids have been reported by the present writers and some of their co-workers. The early studies included a triploid *Gasteria sulcata* X *G. nigricans* (Riley 1948a) and two hybrids between *Gasteria* and *Aloe* (Riley 1947, 1948b, 1950). The *Gasteria* hybrid was received from the Huntington Botanical Gardens and the two intergeneric hybrids from Charles Cass of Pacific Grove, California. No information was available as to whether they were found in the veld or synthesized in the botanic garden, but the second possibility is quite reasonable. In the first plant the percentage of large, apparently viable pollen grains was 46, which seems high for a triploid, but the *Aloe-Gasteria* plants were 100% sterile. In one plant (Riley 1948b) careful studies of chromosome size were made, and they pointed clearly to the fact that two genomes in the hybrid were noticeably different. There must be some genetic similarity between the chromosomes of each genus since there was some pairing at first metaphase. In one plant the long chromosomes ranged from four bivalents in 2% of the cells to eight univalents in 20%, and the short chromosomes ranged from three bivalents (22%) to six univalents (10%). The two genomes differ rather generally, so there was a low degree of meiotic pairing. In the second intergeneric hybrid pairing was somewhat higher.

The senior author studied a collection of 24 species and 10 putative interspecific hybrids of *Gasteria* (Riley 1959d). Analysis was made of meiotic pairing and abnormalities at the microspore division. Of 13 species one was 46% male fertile; all the others were 75-98% fertile, and six of them had 90% or more fertile pollen grains. Of seven hybrids pollen fertility varied from 37% to 80%. He felt that hybridization occurs readily in nature in that genus and that the barriers to hybridization must be weak. Riley and Majumdar (1966b) examined an unnamed variety of *Haworthia limifolia,* which proved to be a hypertriploid with four members each of chromosomes L_2 and L_4. A tendency toward a heteromorphic condition among the trivalents suggests the plant is a hybrid. Majumdar and Riley (1967) made a number of successful interspecific and intervarietal crosses between polyploids and polyploids, and between diploids and polyploids, which shows clearly that there is very little gamete lethality in these plants.

Brinckmann (1960) crossed *Aloe ciliaris* (6*x*) with *A. gracilis* Haw. (2*x*) to produce a tetraploid, *A.* X*lisbonensis* (4*x*), which grows well in Lisbon. It is self-incompatible but can be successfully pollinated interspecifically. This plant indicates that *A. gracilis* and *A. ciliaris* have genomes that strongly correspond structurally.

Intergeneric hybrids have been reported by Sharma and Mallick (1965) a number of times, a fact which the authors state shows "that the assemblage is a natural one." They state also that hybridization at an intergeneric level is successful but that data are few because hybridization is limited by the production of nonviable gametes. These two points seem to be contradictory. They believe that meiosis is generally abnormal and seed formation is scarce,

but these points seem to contradict the observations of other cytologists.

A series of papers by a South African systematist (Bayer 1971a) has recently pointed out several putative hybrids. Much of his work was based on field studies and ecological considerations. He suggests that *H. marginata* commonly hybridizes with *H. margaritifera* and *H. minima* and that there is an extensive *H. margaritifera* x *H. marginata* hybrid swarm between Robertson and Montagu. Also, he considers that *H. poellnitziana,* which occurs at Drew, southeast of Robertson, is probably a hybrid and that in this area also "*H. margaritifera* begins a slow transition towards the smaller more tubercled *H. minima* which is distributed from Cape Agulhas in the southwest to the Gouritz River in the east." He believes, too, that *H. herbacea,* a species with several synonyms, meets *H. reticulata* where the Hex River enters through the Langeberg range and with it forms a hybrid complex that probably involves introgression.

Bayer further states that a hybrid swarm occurs at Wolfkloof (but he has since questioned this statement in a private communication to the present senior author). At the Brandvlei Dam south of Worcester, Bayer considers that *H. schuldtiana* var. *maculata* hybridizes at its eastern limit with *H. herbacea* and that there is confusion at the Wolfkloof site by hybridization with the *H. reticulata* complex. Bayer also cites a hybrid complex involving *H. maculata* and *H. herbacea* (Mill.) Stearn and adds that these taxa are either segregating or introgressing, probably the former. Not only are vegetative characters considered for these taxa (as is usual for systematic studies within the various genera of a whole tribe), but also flower structure, especially flower color and flowering time. The possibility that *H. margaritifera* hybridizes with *H. marginata* in the Bredasdorp area has been suggested by Bayer; such hybridization had caused a number of varieties to be named, but these names cannot be upheld.

Bayer (1970) has recorded several other examples of probably hybrid swarms. Collections of *Haworthia* were made throughout the Robertson Karroo in an area about 80 by 30 km. The most common species there was *H. aegrota* and it was also very variable. In one part of the area there was clearly a hybrid swarm involving that species and *H. reticulata;* nearby another hybrid swarm was present which involved those two species and *H. notabilis*. *H. reticulata* was fairly common there and from the same general region were found *H. aegrota, H. submaculata* v. Poelln., *H. luteorosea,* and possibly *H. guttata* Uit. Bayer believes that all should be regarded as synonyms of *H. herbacea*.

Throughout the Aloineae some of the species seem well established and have an extensive range but some are restricted to small areas. In some geographical regions, as in the Great Fish River Scrub Valley, many species and sometimes many varieties are found, almost as if a wild surge of evolution or a sudden burst of extensive hybridization had occurred. These are undoubtedly areas of hybrid swarms and are similar to some that have been recorded in the Louisiana irises (Riley 1938) and other genera in other families throughout the plant kingdom.

Population studies were made by the present senior author in several regions a number of years ago. They are admittedly crude but were all that it was possible to make at that time and are at least a beginning. Hundreds of plants of *Aloe davyana* were growing on a hillside on a farm at De Wildt in the Transvaal and appeared to be very uniform with little evidence of a hybrid swarm. At the Bushman's River Poort in the Cape Province a number of plants of *Gasteria zeyheri* were examined from a wild and completely uninhabited region. They showed much variability and differed considerably from typical representatives of that species. Other population studies have been mentioned in Chapter 6 in connection with polyploidy.

The various hybrids synthesized in laboratories apart from South Africa show that much hybridization is possible. The putative hybrids and hybrid swarms apparently found in nature indicate that hybridization may have been a greater evolutionary factor in the Aloineae than has often been suspected. Recent intergeneric hybrids have been synthesized by Cutler and Brandham (1977). *Haworthia hybrida* X *Gasteria notabilis, G. planifolia* X *Aloe dorotheae, G. carinata* X *A. dorotheae, G. carinata* X *A. antandroi, G. cheilophylla* X *A. variegata, G. lutzii* X *A. aristata,* and *G. lutzii* X *A. tenuior* var. *rubriflora* were all diploids; *G. dicta* ($2n = 14$) X *A. dawei* ($2n = 28$) was a triploid. *A. dawei* behaved peculiarly at meiosis and often produced aneuploid gametes and gametes with deleted chromosomes or duplicated chromosomes. Sometimes the deletions were large. The cross *G. dicta* X *A. dawei* produced six hybrid offspring. Three were triploids, two were triploids with a large segment deleted from the long arm of one long chromosome, and one was a plant that was clearly hypertriploid with 14 long and 9 short chromosomes.

Brandham (1969a) has emphasized that in the Aloineae species are often separated taxonomically with great difficulty, and he cited Reynolds's (1950, 1966) statements in support of his. He also reaffirmed that the majority of the species are self-incompatible. These two statements are significant in the interpretation of the evolution of the group and substantiate those made since the early days of botanical interest in the tribe. In his analysis of E-type bridges Brandham studied 75 taxa of *Aloe,* five of *Astroloba,* one of *Chamaealoe,* 39 of *Gasteria,* and 41 of *Haworthia* in addition to X*Gastrolea nowotnyi* and X*G. smaragdina* — two intergeneric *Gasteria* X *Aloe* hybrids. He pointed out that the kinds of chromosome changes that result in E-type bridges can occur in a population of a single species but have actually been found in several known hybrids. He believes that cultivated specimens with a high percentage of E-type bridges and univalents should be suspected of being of recent hybrid origin.

The large number of species and varieties, the concentration of many of them in small geographical areas, the small differences that separate many of the taxa from one another, and the great ease of hybridization on both the specific and the generic levels suggest that natural hybridization has been a frequent occurrence in this tribe.

Chapter Twelve

EVOLUTIONARY PROBLEMS

The previously discussed cytogenetic factors operating in the Aloineae are interesting in themselves and provide good material for laboratory exercises in courses in cytogenetics. A very fundamental problem is what role if any these cytogenetic factors as well as other factors play and have played in the evolution of the various taxa of the tribe.

Basic to the question of evolution is the nature of genera, species, and other possible taxonomic categories. Before species can be compared they must be understood so they can be identified and recognized, especially since so much work is done in different countries. Faulty identification can lead to considerable confusion. The correct placing of a given species in a phylogenetic system is hindered if several people use different names for the same species or the same name for different species. Therefore, probably the first problem is taxonomic. Taxonomy is concerned with the identification of the individual plant and is important not only to the theoretical botanist but also, in this example, to the succulent fancier and the commercial grower of succulents.

The identification and recognition of genera is an interesting problem of abstract importance. A preliminary discussion of the genera of the Aloineae was given in Chapter 1 and followed a "splitting" philosophy, listing ten genera. Some authors, however, admit only eight or nine. The ten genera listed here include *Guillauminia* Bertrand with only one species, *G. albiflora* (Guill.) A. Bertrand, and *Leptaloe* Stapf, an endemic genus with six species. In his *Handbuch der sukkulenten Pflanzen* (1954) Jacobsen did not recognize either of these two genera, and he maintained the same position later in *Das Sukkulentenlexikon* (Jacobsen 1970). In both, *Guillauminia albiflora* was retained as *Aloe albiflora* Guill. and the six species of *Leptaloe* were included as *Aloe* L. Section 3. Jacobsen was consistent in his position in these two treatises, but his earlier book was translated into English and published in 1960 by a British publisher. This edition was not merely a translation but a revised and enlarged edition with some new ideas. The authorized translation was made by Hildegard Raabe and supervised by Gordon D. Rowley, who also controlled the nomenclature. Since he is a well-known student of succulents, he undoubtedly exerted considerable influence in determining policy in this revised edition; one might suppose he was the one who de-

cided the independent status of the genus *Guillauminia* which Jacobsen did not use in his first edition and did not include in his *Sukkulentenlexikon*.

Perhaps the most significant problem in the study of evolution in the Aloineae is that of species. What is a species? How many are there? What characters delimit species and determine their identification? The number of designated species has varied greatly as more and more collections have been made and as various taxonomists have revised the genera of this tribe, but whether these species should all be considered as true species is questionable and is regarded differently by different investigators. At any rate, as the senior author has pointed out (Riley 1959d), the whole question of evolution in the tribe probably cannot be solved until the taxonomic problems are better clarified.

The problems of identification and plant names can be approached either by the use of conventional "classic" methods or by the methods of the newer numerical taxonomy. With either method there are certain inherent difficulties. Some of them were discussed in Chapter 3 and need only be mentioned here. With the former, basically the same methods are used with the Aloineae as are used in other genera and families of plants. Plants are collected, preferably from the wild, and are pressed and mounted. Labels are attached giving the name the collector has used in identifying the plant; information as to habitat, geographic location, and perhaps ecological data; a number under which it can be filed; the date of collection; and the name of the collector. This is the ideal situation, but with the Aloineae all too frequently (in older collections especially) most of this information is missing. Also the nature of the plants is such that good herbarium specimens cannot be prepared. The vegetative parts of the plants are thick and succulent, and are bulky in the press. Not only are they difficult to press, but they are sometimes difficult to kill; occasionally herbarium specimens continue to grow in the press for weeks and months. The result, when they are finally removed from the press, is a stem with many elongated, etiolated leaves which have continued to grow from the base. However, in spite of the difficulties involved and the difficulty of comparing specimens, no satisfactory substitute for herbarium specimens has been devised, although a series of clear photographs might well accompany (but not replace) the herbarium specimens.

This problem with herbarium specimens is not new and was recognized by Burchell (1822) over 150 years ago. Much more recently Bayer (1976) emphasized the importance of herbarium specimens. He pointed out that von Poellnitz, who did such extensive collecting in the early part of the twentieth century, studied living material and did not leave many herbarium specimens. He may have had a small private herbarium since some herbarium sheets were found in his castle after his death. His collection of live plants had previously been given to the Berlin-Dahlem Botanical Garden, but apparently the Garden did not press and preserve them. (Dr. von Poellnitz was killed less than a year before the end of World War II when a pilot mistook his castle for an industrial plant and bombed it.)

TABLE 12.1 Number of Species, Varieties, and Hybrids of Aloineae Listed by Jacobsen (1970)

Genus	Species	Varieties*	Hybrid Species	Hybrid Varieties
Aloe	384	80	31	1
Astroloba	13	7	0	0
Chamaealoe	1	0	0	0
Chortolirion	5	0	0	0
Gasteria	72	27	9	0
Haworthia	157	239	6	0
Lomatophyllum	12	0	0	0
Poellnitzia	1	1	0	0
Total	645	354	46	1

*This column also includes forms, mutant forms, forms of varieties, mutant forms of varieties, and subvarieties.

One important source of confusion in identifying these plants is that many species were named a century or two ago when descriptions were not as carefully written as they are today. The early type specimens show bad faults such as careless preparation, inadequate material collected, and insufficient habitat information. Frequently in the early work the only geographic location given was "Cape Colony" or "South Africa," so the actual habitats cannot be identified. This difficulty is compounded by the fact that many of the normal "species" consist of only one clone and therefore can be located with only the greatest difficulty if at all. Another problem is that herbarium specimens of many of the early types were placed in European botanical gardens (because they were collected by European botanists) and therefore are not readily accessible to South African (or American) botanists. Furthermore, many type specimens have been lost over the years, especially those that succumbed to fire during the bombing raids of World War II. Because of these factors the comparison of material with the type specimens or even with nontypes that were studied by other research workers is a discouraging proposition.

The number of species, varieties, and other taxa of subspecific rank is large, even disregarding synonyms and invalid names. The number varies greatly among the different genera, as becomes startlingly clear when Table 12.1 is examined. *Aloe* and *Haworthia* are large; *Gasteria* is moderately large; *Astroloba, Chortolirion,* and *Lomatophyllum* are small; and *Chamaealoe, Guillauminia,* and *Poellnitzia* are monotypic. (Since *Guillauminia* and *Leptaloe* were not included in Jacobsen, they have been omitted from Table 12.1.) If varieties and other taxa of subspecific rank are added to the list, over 1,000 species and other categories have been identified. By far the largest number of varieties is in *Haworthia,* where it greatly exceeds the number of species. Relatively large numbers of varieties, although fewer than the number of species, are found in *Aloe* and *Gasteria*.

Interspecific and intervarietal hybrids are infrequent although more numerous than in many genera in other tribes and orders (Table 12.2). They have been discussed in

Chapter 11 and add confusion to any taxonomic analysis of the Aloineae. In addition there are some intergeneric hybrids. Since much of the Aloineae material was obtained from botanical gardens, the origin of many of the hybrids cannot be determined. Were they the result of accidental self-pollination in a garden or greenhouse abounding in insects? Were they artificial hybrids produced by a gardener or a geneticist? Were they natural hybrids collected in the wild? Frequently no evidence is available on these points. While sometimes interesting for cytogenetic studies, such putative hybrids should not be used to build a phylogenetic sequence or for any kind of evolutionary significance. The widespread presence of such hybrids complicates greatly our understanding of the nature of species, but hybrids undoubtedly exist in the wild state — unless the evidence and descriptions of such hybrids by competent and experienced taxonomists over 200 years can be ignored. In a few instances the hybrid nature of some plants is clear, as with the *Aloe arborescens* X *A. chortolirioides* hybrid of Müller (1945), which was growing near the habitats of the two parents. With so many species distinguished from one another by only small differences, with so many species apparently very narrowly distributed so that some may consist of only one or two clones, and with so many interspecific and intergeneric hybrids, the problem is very complex and a definition of a species is difficult.

Another confusing feature of the Aloineae is the morphological differences that occur between juvenile and adult forms of the same plant, as has been observed by several students of the Aloineae and emphasized particularly by Schelpe (1958) in his excellent population study of *Gasteria*. Unfortunately his paper is in an obscure publication and thus not well known outside South Africa. He pointed out that the arrangement of the leaves is sometimes distichous and sometimes spiral or multifarious; some plants such as *G. pillansii* have a distichous arrangement throughout their life, whereas aloes and many other gasterias are distichous when young and spiral or multifarious if they mature in a favorable environment. Environmental conditions can also affect this leaf arrangement. The distichous arrangement of the juvenile forms of the *G. maculata* complex is retained in the plants of a population that are heavily shaded by surrounding bushes; those in exposed situations become spirally or multifariously arranged early in their development. In a drier area of this complex most plants have a spiral leaf arrangement and usually only seedlings are distichous; adult plants growing in very deep shade may have a weakly spiral arrangement. Aloinologists not familiar with the effects of age, sunlight, and moisture on the plants of this group are inclined to be led into errors. Additionally, single specimens of several of these forms differing in age and environmental development that were distributed to several botanists outside Africa probably have been described and named as several different species.

Sun and temperature have a considerable phenotypic effect on some species. Some years ago the senior author came upon a group of several plants of *Aloe mitriformis* growing on the side of a steep hill about 12 meters above

TABLE 12.2 Hybrid Genera of Aloineae Listed in Jacobsen (1970)

Hybrid Genus	Putative Parents	Hybrids	Varieties
X*Gasterhaworthia* Guill.	*Gasteria* X *Haworthia*	3	0
X*Gastrolea* E. Walth.	*Gasteria* X *Aloe*	18	1
X*Gastrolirion* E. Walth.	*Gasteria* X *Chortolirion*	1	0
X*Lomataloe* Guill.	*Lomatophyllum* X *Aloe*	1	0
X*Lomateria* Guill.	*Lomatophyllum* X *Gasteria*	1	0
Total		24	1

the road that went through the old Franschhoek Pass. Plants growing in full sunlight were a deep, rich red in color, whereas those growing in the shade under various shrubs were green. Sun and shade forms of the same species sometimes vary so greatly in other characters as well as in color that they may not even be recognized as members of the species. Schelpe (1958), for example, studied the *Gasteria obtusifolia* complex and found that plants growing in extreme shade were different from those growing in full sun and were similar to illustrations that had been published of *G. lingua*. Bayer (1970) writes that in *Haworthia reticulata,* plants growing during a hot dry summer develop very beautiful shades of red whereas plants in winter are a bright green. These environmental differences can easily confuse a taxonomist.

The importance of the recognition of juvenile forms is well illustrated by a study of *Gasteria beckeri* and related species. Schelpe (1958) found a colony of *G. beckeri* near Port Elizabeth. They were typical smooth-leaved adults, but around their bases were many young suckers which showed wide variations in the characters of their leaves. The leaves of the older suckers were coarsely tuberculate and spirally arranged and resembled plants of another species, *G. stayneri*. On the other hand, the younger and smaller suckers were distichous, had finely tuberculate leaves, and resembled many plants of a third species, *G. armstrongii*. In a thorough study of the situation Schelpe found that when the *G. beckeri* plants developed, three main changes occurred in the leaves, and they did not take place at the same rate: (1) a distichous to a spiral and multifarious leaf arrangement; (2) a densely and finely tuberculate leaf to a leaf that has coarse, widely spaced tubercles and finally a leaf that is spotted in different ways; and (3) an oblong leaf with an obtuse, mucronate apex to a concave triangular leaf with an acute apex. Schelpe found that *G. armstrongii* seems to occur in the driest parts of the complex, *G. beckeri* in the moister parts, and *G. stayneri* in intermediate regions. He further suggested that *G. stayneri* and *G. armstrongii* are perpetual juvenile forms of *G. beckeri* that appear as they do because of the environmental conditions under which they develop. He also found that Salm-Dyck's illustration of *G. decipiens* could be that of an "overfed and thoroughly turgid *G. beckeri*" and suggested that *G. decipiens* Haw. is the correct name for the *G. beckeri* complex. If so, *G. stayneri* and *G. armstrongii* denote merely habitat forms of that species. This intensive study illustrates the futility of using names attached to plants

in greenhouses on other continents, especially those that have been out of South Africa for a dozen or more years.

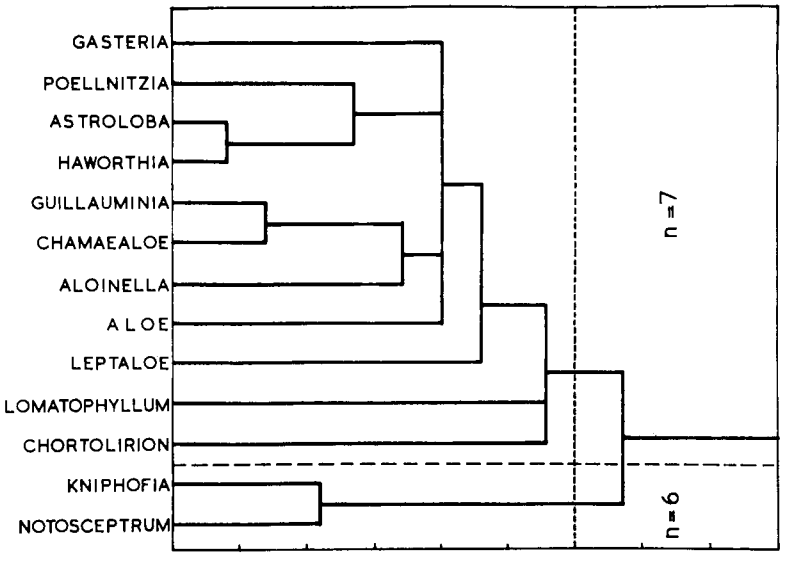
The methods of the newer, numerical taxonomy have not yet been applied extensively; Rowley (1967, 1969) used them to compare the ten genera of the Aloineae listed in Chapter 1 with *Aloinella, Kniphofia,* and *Notosceptrum* — all of which he includes together for study and comparison.

Aloe haworthioides Bak. is included with *A. andringitrensis* Perr. and *A. sempervivoides* Perr. in Section 1 Aloinella of the genus *Aloe* in Jacobsen (1960), which is the second edition of his *Handbuch* as translated by Raabe and Rowley. In *Das Sukkulentenlexikon* all three species are included in Section 1 Aloinella with *A. albiflora* Guill., *A. bellatula* Reyn., and *A. perrieri* Reyn.; but *A. sempervivoides* Perr. is given as a synonym of *A. parvula* Bgr. They are all from Madagascar and therefore not included in Reynolds's (1950) first book but are in Reynolds's (1966) second. Reynolds in this latter work corrected the nomenclature of the last two species. *A. perrieri* Reyn. was considered by Perrier to be *A. parvula* Bgr.; more recent studies showed that *A. sempervivoides* Perr. was the true *A. parvula* Bgr., so the name of *A. sempervivoides* now becomes a synonym of *A. parvula* Bgr. This meant that the species Perrier found and regarded as *A. parvula* had to be renamed, so Reynolds renamed it *A. perrieri* Reyn. In the present work these six species have been placed under *Aloe,* not under the genus *Aloinella.*

Using numerical taxonomy Rowley (1967) arranged the genera of the Aloineae — including *Aloinella, Kniphofia,* and *Notosceptrum* — in a phenetic diagram. *Kniphofia* and *Notosceptrum* are together. This is logical, and they should be separated into a different tribe, as Hutchinson (1944) did. Not only are they apart from the other genera on the phenetic diagram but they are also different cytologically. In these two genera $n = 6$, and in the others $n = 7$. Omitting *Aloinella,* which the present authors prefer to place under *Aloe,* the ten genera of the Aloineae fall into the grouping as shown in Figure 12.1. This grouping except for *Lomatophyllum* is very similar to the phenetic diagram published by Rowley (1969) on the basis of the classic or, as he calls it, "intuitive" method of taxonomy.

In assessing the factors that possibly influence the evolution of the group, it seems convenient to divide them into the conventional problems of inherited and environmentally imposed factors.

The basic karyotype has been known for over 50 years and is so remarkably constant throughout the genera that there apparently is no established deviation. Occasional plants have heteromorphic chromosomes as the result of translocation, and a few show the centric fusion type of segmental interchange, but these deviations do not appear in the homozygous state and are perpetuated only in individual vegetatively-reproducing clones. Even the centric-fusion Robertsonian type of translocation does not produce a change in the chromosome number as it does in *Drosophila.* Pericentric inversions (as well as paracentric ones)



12.1 Phenetic diagram showing relationship of the genera of Aloineae and Kniphofioideae. (*Taxon*, vol. 18)

occur rarely in this tribe. If they occur, pericentric inversions may change the karyotype by converting an acrocentric chromosome into a metacentric one or vice versa, but there is no evidence that these changes are inherited in this group because the few that are found are in the heterozygous state. Deviations from the standard Aloineae karyotype are few in number and seem never to be homozygous, so their occurrence is inconsequential.

Deviations in chromosome number from that of the basic karyotype are also few. An occasional aneuploid has been found but none was homozygous, and no diploid species or variety with a chromosome too many or too few has even been seen. The few aneuploids that are known are in polyploid plants of *Haworthia*. Aneuploidy simply has had no significant effect in establishing new species or varieties (Riley 1968).

Polyploidy has been a puzzling factor in the Aloineae and varies greatly in amount in different genera. It is relatively infrequent in *Aloe* and *Gasteria* and has not been found in a number of sections of *Haworthia*. However, in this last genus there is a very high percentage of polyploidy in the Coarctatae, and Tessellatae sections and a sporadic one or a few in the Arachnoideae, Limifoliae, Loratae, Margaritiferae, Retusae, and Trifarieae. An occasional horticultural variety and/or hybrid is also a polyploid. From evidence available so far, polyploidy is not a species former, although it may account for some of the varieties found within certain species. Some species such as *Haworthia baccata, H. coarctata, H. herrei, H. reinwardtii, Gasteria maculata, G. nigricans, G. spiralis, G. subverrucosa, G.* X*cheilophylla, Aloe humilis, A. tidmarshii, Astroloba pentagona,* and a *Gasteria* X *Aloe* hybrid

contain both diploid and polyploid forms. Why polyploid forms arise, why they do not arise more frequently, and why they are so numerous in certain sections of *Haworthia* and not in others are questions still to be answered. One possibly important point is the correlation in *Haworthia* of sections containing species with numerous varieties and a high incidence of polyploidy. The correlation is not perfect but is higher than expected; it is especially true of *H. reinwardtii* and *H. tessellata*. Perhaps these varieties arose largely as allopolyploids with enough hybridization to produce morphological varieties. Many of these varieties and polyploids have been collected under natural conditions and are not the products of cultivation.

Brandham (1971) suggested that the higher percentage of polyploids in the Coarctatae and Tessellatae sections may result from a greater variation of these sections under wild conditions, but he believes they more likely have been caused by the selection of polyploids during a long period of cultivation. However, the present authors' plants have fairly recently been collected in the wild and have not had a long history of cultivation; this evidence would not support Brandham's thesis. Furthermore, the basic problem is the evolution of plants in nature, so cultivated plants are of little importance.

Translocations may often lead to changes in chromosome length. However, the differences may often be slight and the lengths that are exchanged may be so nearly the same that very exact and careful measurements must be made to show any real differences in chromosome length among the many species. Sharma and Mallick (1965) have recorded many such differences, which were revealed by a special pretreatment technique that apparently is necessary to reveal these minute differences. Those authors assume that these differences have been important in the evolution of the tribe, stating: "The present investigation, however, shows that in addition to gene mutation, structural changes involving, principally, rearrangements of chromosome parts have played a very important role in evolution."

The significance of that study might well be examined critically. Sharma and Mallick give chromosome measurements for 40 species and varieties of the Aloineae belonging to three genera, grouping the various chromosomes into twelve types and subtypes. Assuming that the measurements are accurate (and they should be, since they have been made by very careful workers), are they relevant? As the present authors understand the situation, each measurement was made at one time on one clone. This point is understood from Sharma and Mallick's statement: "Though a large number of individuals of each species were studied, all of them evidently belonged to a single clone." It makes little difference how many plants were studied if they are all the same, unless differences in time are considered. For significance, Sharma and Mallick should report more than one determination for each species. They should study many clones of the same species from several geographic locations (if they exist). For as detailed a study as they were making they should study the chromosomes of more than one root tip in a plant; this point would not be important for a study of chromosome numbers

but should be for a comparison of species involving such small differences in measurements. They should also study chromosomes of the same plant at different times of the year and in different years; again chromosome numbers should be less affected than small differences in measurements. What results have they found? Sharma and Mallick state: "Certain differences are noticeable which distinguish one species from another." This statement would be more accurate if "clone" were substituted for "species."

A complicating factor in the evolution of the Aloineae that does not exist in many other plants is the extensive reproduction by vegetative means. This method of reproduction results in clones often extending over half a meter square in *Haworthia* and involving perhaps as many as ten or twelve side branches. It is a widely used method of reproduction among succulent fanciers or dealers and is common in nearly all species except a number of aloes. In *Gasteria,* leaf cuttings are a useful method of greenhouse propagation, producing perhaps eight or ten little buds at the soil level if the cut surface of the leaf is placed in sand. It is not, however, the sole method of reproduction, as Sharma and Mallick (1965) imply. They emphasize gamete sterility and seem to feel that "most species reproduce principally by vegetative means, and continued cultivation under horticulture has possibly eliminated their capacity for sexual reproduction. Meiosis is abnormal for nearly all the species where it could be studied and seed formation is scarce." These statements are astonishing for several reasons. If sexual reproduction has been eliminated or largely eliminated by continued cultivation under horticulture, it has nothing to do with the problem of evolution — which means evolution in nature, not in the laboratory. What happens in the botanical garden should be disregarded and is another reason for not trying to base theories of evolution on botanical garden collections. Vegetative reproduction is very prolific in the Aloineae in general but has not eliminated sexual reproduction.

Sharma and Mallick's observations on fertility do not agree with those of the present authors. In *Gasteria,* for example, Riley (1959b) studied 13 plants and found the range of pollen fertility to be from 46% to 98% with six plants having at least 90% fertile pollen. In *Haworthia* 147 diploid plants were studied (Majumdar and Riley 1973); of these, 72 — almost half — showed pollen fertility of 90% or better and only 11 plants were less than 50% fertile. These figures hardly substantiate the theory of Sharma and Mallick.

Sharma and Mallick's data do not seem to support their own statements: "Under conditions of cultivation, sexual reproduction is nearly obsolete, vegetative means of propagation being obligatory." That statement presumably implies that meiosis is abnormal. Yet in the descriptions of six of their 40 species they furnish some information on fertility as follows: *Aloe spuria,* "The meiotic stages are more or less regular"; *A. microstigma,* "Meiotic behaviour is more or less normal"; *A. variegata,* "In most cases, meiosis is found to be normal"; *H. fasciata,* "Meiotic behaviour is mostly normal with seven bivalents"; *H. planifolia,* "In meiosis, seven bivalents have been ob-

served. Irregularities...are also seen"; *G. minima*, "Meiotic behaviour is normal showing seven bivalents in metaphase I."

Since so much evidence has been found to disprove the idea that the Aloineae — at least after a long period of cultivation — are highly sterile, it is interesting to speculate on the origin of that idea. Plants growing in a greenhouse rarely set seeds, but not because they are sterile. As mentioned in Chapter 10, the plants of these species are highly self-incompatible, almost never setting seed with their own pollen. Because the average greenhouse has few insects and ideally should have none, cross-pollination and cross-fertilization rarely occur. Therefore, the chance of compatible pollinations is small, and the almost total absence of large fertile capsules is readily explained. The presence of self-incompatibility in the Aloineae was reported years ago by Marshak (1934), Sears (1937), and others and more recently by Riley and Majumdar (1966a), Riley, Majumdar, and Hammack (1967) and Brandham (1969a).

If chromosome mutations such as polyploidy, aneuploidy, and intrachromosomal aberrations have not been impelling instruments in the development of new species, two more agents might be mentioned — gene mutation and hybridization. It is difficult to assess the consequences of gene mutation since few genetic studies have been initiated. The reason is clear. While the Aloineae are ideal for chromosome studies, they are less valuable for genetic studies. Crosses are made with ease but development from seed to seed requires two or three years and many crosses cannot be made because of self-incompatibility. That is not to say that genes are unimportant, for they were undoubtedly a significant factor in the evolution of the tribe, since many different phenotypes have arisen. The possible importance of genes in this tribe has been suggested by several authors in the past (Riley 1959d; Sharma and Datta 1962; Sharma and Mallick 1965; Riley 1968); it probably cannot be minimized, but it has not been proven.

Hybridization is extensive in the Aloineae and has probably played a significant role in evolution. Hybridization occurs between species and between genera to such an extent that some students of the group have wondered whether perhaps there is only one real genus in the Aloineae. Hybridization has compounded to a great extent the problems of taxonomy, but reduction of all genera to one would not simplify the question. Hybridization is so extensive that it cannot be ignored as a foremost factor in the evolution of the tribe.

Not only is interspecific hybridization found on a large scale, but intergeneric hybrids are not rare. Several are listed by Jacobsen (1970), and Rowley (1967) includes 12 hybrid "genera" in his list of intergeneric hybrids: *Alchamaloe (Aloe* X *Chamaealoe), Aleptoe (Aloe* X *Leptaloe), Allauminia (Aloe* X *Guillauminia), Aloella (Aloe* X *Aloinella), Aloloba (Aloe* X *Astroloba), Astroworthia (Astroloba* X *Haworthia), Gasterhaworthia (Gasteria* X *Haworthia), Gastrolea (Aloe* X *Gasteria), Gastrolirion (Chortolirion* X *Gasteria), Leptauminia (Guillauminia* X *Leptaloe), Lomataloe (Aloe* X *Lomatophyllum),* and *Lomateria (Gasteria* X *Lomatophyllum).* Some have been known for 30

or 40 years, but several were published for the first time in Rowley's paper.

These intergeneric hybrids throw some doubt on the validity of the original genera and suggest that, since they are not separated by any reproductive barriers, they are all species of one vast genus, perhaps *Aloe*. This suggestion is by no means either new or unique and was first proposed about a hundred years ago by Salm-Dyck. Rowley (1967) commented on the idea:

> Having set aside *Kniphofia, Notosceptrum* and *Chortolirion* as well-founded genera, the remaining ten taxa constitute what we would call a "critical" group in which no evolutionary pattern or breakdown into units is apparent. The only consistent treatment would be to reunite them all as one genus, or to multiply the genera *ad lib.* to cover all possible permutations of characters. Neither extreme could be recommended for a general purpose classification where identification, retrieval of information and similar practical issues are of prime importance.

It seems to the present authors that in spite of the fact that taxonomy and evolution in the Aloineae have been studied for many decades, the complete information necessary to formulate an adequate theory to account for the origin of the various genera, species, and varieties is not yet at hand. Many approaches are necessary, no one of which will produce all the answers.

Karyotype analysis does not provide a perfect solution in spite of Sharma and Mallick's (1965) suggestion that very small differences in chromosome length adapt each of the hundreds of different species to different ecological niches. This is an attractive theory, but it requires the presence of almost a thousand different microhabitats, and their existence has never been demonstrated. The reasoning seems to be that each slight karyotype difference is adapted to a different habitat. There are hundreds of minute karyotype differences. Therefore, there must be hundreds of different microhabitats to which presumably the various species are adapted. Therefore, karyotype differences are the factors that determine evolution and adaptation in the group. The microevolution problem is fascinating, but why do some species such as *Aloe ferox* and *A. marlothii* occupy vast areas whereas others, including many of the haworthias found in the Fish River Scrub Valley, seem to consist of only one small clump? If a species occupies a large area, it is difficult to suppose that the entire area is uniform, that the whole region is one large microhabitat, and that there are no small differences such as those that would account for microevolution. The alternative explanation seems to be that some species are adapted to only one very restricted habitat whereas others cover large territories with many habitats and have a great adaptive range. The idea of Sharma and Mallick would be more attractive if those writers could demonstrate that these different habitats exist. So far the problem is purely speculative.

The need for field studies is great, as is the need to minimize if not completely disregard studies of the Aloineae based on collections made 50 or more years ago which

have been largely unattended since then. Evolution may occur in greenhouse and botanical garden collections, but the only evolution of real significance to a biologist is that which occurs in the field. Therefore, all chromosome data based on collections made over a dozen years before the plants were studied and not based on plants recently obtained from South Africa should be depreciated.

The more the importance of greenhouse studies is minimized, the more field studies must be emphasized, but they should be studies from both the morphological and cytological aspects. Such studies have been too few so far. The tendency of the earlier taxonomists was to collect widely and extensively but not to cover any given region intensively nor to study populations, largely because the population concept was not understood or recognized at that time. The early cytological studies were based on botanical garden collections in Europe and Japan and had no connection with natural populations.

Several South African students of the Aloineae have recognized that a real solution to the evolutionary problem in the group will come only from field and population studies. Smith (1948), in an article on the naming of haworthias, mentioned that in that polymorphic genus, field studies are necessary and without them many of the previous studies had only contributed to a very confused picture. He pointed out that if plants are shipped on a long journey, as to a taxonomist in Europe, the innermost leaves sometimes elongate during the trip, causing the plants to vary considerably from their original appearance. The fact that they frequently elongate in a plant press was stated earlier in this chapter. Smith also mentions that some of Resende's species and varieties were named from garden plants or from seed from unspecified localities. He believes that no new species or varieties should be named if only one or two plants are available for study, and strongly suggests that new species and varieties should be described only by someone who knows the plants in their natural habitats in South Africa and can appreciate their variability in the wild. Bayer (1971b) points out that Berger in *Das Pflanzenreich* (1908) has some interesting comments on garden hybrids; Bayer adds that many new species today are recognized that were not based on wild populations; in fact, Bayer writes, there has been a complete failure on the part of haworthia students to signalize how important locality and variability can be. The same statement would apply to other members of the Aloineae as well; they have never been studied on a population basis.

Fortunately botanists who are in a position to correct this situation have recently begun to recognize the importance of the problem. Brandham and Johnson (1977) studied a small population of *Haworthia browniana* that was growing on the Lelie Krants farm near Uitenhage outside Port Elizabeth in the southern part of the Cape Province. In an area 50 meters square, nine plants were examined from as far apart as possible and each one possessed the interchange that was described in Chapter 9. All the plants were diploids and each was heterozygous for the interchange.

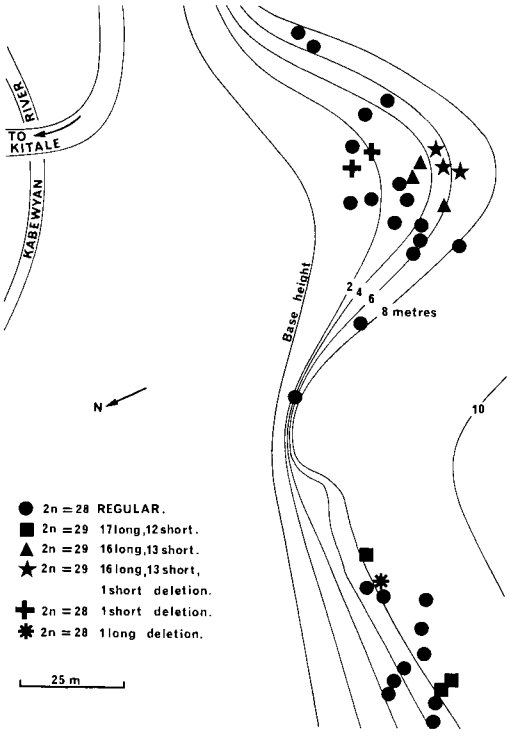
An interchange of the centric fusion type was found by

Brandham and Johnson (1977) in *Aloe rabaiensis* and is described in Chapter 9. Data were obtained from 62 plants, all of which were diploids but eight of which carried the translocation in heterozygous condition. These plants were growing in mostly dense scrub in a strip about 20 meters wide just west of the Nairobi National Park in Kenya. There were two collections of this population. The first was really a sample and showed one interchange out of five plants. The general area was roughly in the shape of a 60° triangle. If it is pictured as lying on its long side with the short side extending up on the right, two interchange plants were located at the extreme left end, one at the right angle, and four at the angle formed by the right leg and the hypotenuse. The normal plants were rather well distributed throughout the area.

Another detailed population study was one of *Aloe pubescens* Reynolds. This species has been found in only one locality, situated along a stream that crosses the road from Shashamane to Addis Ababa in the Arussi Province of Ethiopia. Brandham and Johnson (1977) examined 31 plants from this region; all were diploids but two carried an interchange in which pieces of an L_1 and an L_2 chromosome had exchanged places as described in Chapter 9. The plants were growing for about 600 meters along the banks of a stream through grassland with a few trees. The two interchange heterozygotes were growing over 200 meters apart; one was on the edge of a cliff about seven meters high.

An even more exciting population study is Brandham and Johnson's (1977) detailed analysis of the plants of *Aloe elgonica* Bullock which were growing on the lower eastern slopes of Mt. Elgon. The southern and eastern slopes of this mountain on the Kenya-Uganda border are the only place where this species occurs. This population is the most complex that has been studied to the time of this writing. A preliminary survey was made in 1973 by Brandham; two populations were sampled and ten plants of each were studied. At the first site all ten plants were normal tetraploids, but in the second, which was very near the first, five of the plants had a deletion in which most of the short arm of one of the short chromosomes was missing. One tetraploid had one deleted short chromosome and another had two; three plants were aneuploids and had the deleted short chromosome plus twelve normal ones. No detailed records were obtained of the precise location of any of the plants in the general area; this second site was revisited in 1974-75 so that a more detailed analysis could be made.

The second site included a slope facing north and a second slope further east. The second slope was amphitheaterlike and the two slopes were separated by a short region of almost vertical rock. Fourteen plants were studied from the first slope, 23 from the second, and one from the rock between them. Two kinds of deleted chromosomes, one short and one long, were found and have been discussed in Chapter 9 above. On the first slope there were ten normal tetraploids, three tetraploid-aneuploids ($2n$ = 17 long and 12 short), and one tetraploid with the long deletion. On the amphitheaterlike slope there were 15 normal tetraploids, three tetraploid-aneuploids (16



12.2 Map of the second site of the *Aloe elgonica* plants studied by Brandham and Johnson (1977). (*Plant Syst.Evol.*)

long and 13 short), three similar tetraploid-aneuploids with a short deletion, and one tetraploid that was normal except that it carried a short deletion. The single plant on the rock between the slopes was a normal tetraploid. The distribution of the plants is shown in Figure 12.2. The plant with two deletions that was found in 1973 was not found on the second excursion to the territory.

A study of the figure shows a peculiar situation: none of the aberration types occurs on both slopes. Brandham and Johnson believe that the long deletion aberration on the north-facing slope is the result of a single chromosome mutation and that it is probably of very recent origin. On this same slope, one aneuploid plant is separated widely from the other two, which are close together and may represent a single clone. On the amphitheater slope, the aneuploid with an extra short chromosome, the similar aneuploid with the short deletion, and the tetraploid with the short deletion are all found in close proximity. Each type consists of two or three plants which extend downhill; Brandham and Johnson explain this pattern by assuming that tall stems may rot at the base or otherwise become dislodged and can then roll down until they lodge in the rock further down, where they reroot and become established. The juxtaposition of these three aberrant types, according to those authors, shows that plants with the extra short chromosome with or without the deleted chromosome can breed with one another and actually

do so. The restriction of the aberrant types to one or the other of the populations on the two slopes, as Brandham and Johnson show, indicates that the spreading of the two chromosomal types, the extra short chromosome and the short arm deletion, is very limited sexually.

Brandham and Johnson (1977) have augmented their cytological and population studies with some interesting generalizations. Crosses among plants of *Haworthia browniana* all failed to set seed; since this is a self-incompatible species, all the plants of that species are probably members of a single clone. In a sexually reproducing species such as *H. reinwardtii* var. *chalumnensis,* if all the plants are interchange heterozygotes, probably either selection favors the heterozygote or the heterozygote is better adapted than the homozygote under environmental stress (Brandham 1974). Centric fusion homozygotes, if they occurred, would represent a new karyotype, but they have never been found. When the homozygotes do not occur it is possible that the dissemination of the heterozygotes is purely vegetative. As Brandham and Johnson show, no two species in the Aloineae are known that differ from one another by homozygous interchanges. Probably natural selection eliminates possible homozygotes. Apparently the bimodal karyotype has advantages that the homozygous condition of any major structural change cannot improve (Brandham and Johnson 1977).

Recapitulation

The problem of genera, species, varieties, and other taxa in the Aloineae is compounded by a series of factors that have been discussed in various places in this book but have never been brought together. It seems advisable at this point to assemble them in a discussion of the whole problem. The factors can be divided for convenience into those intrinsic to the plants and those arising largely from the methods used by the various taxonomists who have studied the tribe.

Factors Intrinsic to the Plants

1. Variation; it is considerable since there are many slight differences:
   (a) Genetic; not many genes have been identified.
   (b) Environmental; important in some species.
   (c) Resulting from the age of the plant; very important in some species; can easily lead to the confusion of species.
2. Natural hybridization:
   (a) Interspecific hybridization; apparently common.
   (b) Intergeneric hybridization; fairly common between some genera.
   (c) Hybrid swarms; several found but much more study needed.
3. Self-incompatibility; makes cross-fertilization obligatory between some combinations of crosses.
4. Vegetative reproduction; encourages persistence and multiplication of various types and of heterozygotes.
5. Chromosome aberrations; not important in the evolution of the group:

(a) Polyploidy; has apparently not been an important factor in speciation.
(b) Aneuploidy; found only in heterozygous condition; found only in polyploid plants; not an important factor in the evolution of this tribe.
(c) Deletions; only found in heterozygous condition and in polyploids; not important.
(d) Duplications; not an important evolutionary factor in the Aloineae.
(e) Inversions; of limited importance in the Aloineae; all known are heterozygous.
(f) Translocations; all that are known are heterozygous; the centric fusion type does not change (reduce) the chromosome number in the Aloineae.

Factors Appertaining to Taxonomic Methods

1. Material collected from wild plants:
   (a) Often no location or only a vague one given.
   (b) Often a species or variety based on only one plant.
   (c) Many (especially early) collections extensive but not intensive.
   (d) Sometimes no herbarium specimens made.
   (e) Some plants named by amateur botanists.
   (f) Some plants named by succulent fanciers or dealers who are interested in naming new species even when they do not really exist.
2. Botanical garden collections:
   (a) Many taxa named from plants growing in botanical gardens.
   (b) Plants subject to mechanical errors such as switched labels and to general neglect.
   (c) Often no data as to the source of origin of a plant or no data showing when it was received by the garden.
   (d) Plants growing in gardens for many years may develop somatic mutations and appear different.
   (e) Some may be artificial hybrids but not so designated.
   (f) Some plants grown in gardens may have been raised from seeds.
   (g) Plants received as seeds from elsewhere may not be true to their parental types.

The Aloineae are an exceptionally difficult group of plants taxonomically and the problem has not been helped by the earlier taxonomists. Probably the most logical and most satisfactory solution would be to declare all previous epithets null and void and to begin all over; that, of course, is impossible. Perhaps, at least, all eighteenth- and nineteenth-century names should be seriously re-evaluated unless the plants were named in South Africa by botanists who were acquainted with them under field conditions where their natural variability could be understood. Bayer (1976) in his excellent and very recent handbook of *Haworthia* recognizes this view and suggests that none of Resende's names can now be upheld as species. Perhaps Resende's most interesting specific epithet is *Haworthia lisbonensis* Res. which was named from the place where Resende found it — the Lisbon Botanical Garden. Also, all

determinations of chromosome numbers should be regarded with suspicion if they were made upon plants in botanical garden collections of long standing. This situation should not apply to plants sent from South Africa in recent years for cytological purposes.

The recent studies of Bayer and Brandham and of Schelpe's earlier paper are a preview of the kind of detailed field studies that will be necessary if any effective solution to the taxonomic problems in the Aloineae is to be found. Field studies are necessary for sound taxonomic work in this tribe; field studies plus ecological and chromosomal studies are requisite for a knowledge of its biosystematics and evolution.

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Index of Families, Genera, & Species

Species mentioned nowhere but in the discussion of geographical regions (Chapter 2) are not included in this index. F: figure; T: table.

General Index

The Authors

Herbert Parkes Riley, Ph.D., D.Sc. (Hon.), is Distinguished Professor Emeritus, University of Kentucky, and Adjunct Professor, Transylvania University.

Shyamal K. Majumdar, Ph.D., is Associate Professor of Biology, Lafayette College, Pennsylvania.