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COMPARATIVE ANATOMY OF HAWAIIAN PEPEROMIA (PIPERACEAE) SPECIES.

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COMPARATIVE ANATOMY OF HAWAIIAN PEPEROMIA (PIPERACEAE) SPECIES

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY

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by

Setijati Notoatmodjo

Dissertation Committee

Beatrice H. Krauss, Chairman
Gladys E. Baker
Maxwell S. Doty
Douglas J. C. Friend
Hans R. Hohl
Charles H. Lamoureux

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ABSTRACT

In an effort to interpret the trends of specialization within the genus Peperomia, carpel morphology, meiotic chromosome number, meiotic irregularities, mode of leaf arrangement, and leaf and stem anatomy of 26 Hawaiian species of Peperomia have been studied.

Based on the available data, the following trends of specialization may have occurred in the genus:

1. There has been a reduction in the number of carpels composing the gynoecium. A tricarpellary gynoecium has given rise to a bicarpellary, and finally to a monocarpellary gynoecium. Such a trend is demonstrated by a reduction of the number of stigmas, as well as by a reduction in number of dorsal carpellary bundles in the ovary wall.

2. The basic chromosome number of the Hawaiian species of Peperomia is n = 22, and one species has a variation of n = 24. Polyploidy has been observed, and numbers of n = 44 and n = 48 have been recorded. The presence of meiotic irregularities and polyploidy suggest the possible roles of hybridization and polyploidy in speciation within the genus. The possible role of alteration of chromosome number in somatic cells in the speciation of the Hawaiian species is discussed.

3. Whorled leaf arrangement in the genus Peperomia has been derived from a spiral phyllotaxy. It is suggested that the whorled arrangement was

brought about by three factors, i.e., the failure of internodes to elongate, the lateral fusion of leaf primordia, and the fusion of the neighboring leaf traces. The failure of internodes to elongate is due to reduction of both cell division and cell elongation.

4. In this genus, vascular bundles are scattered in the pith resembling those of monocotyledons. Secondary growth has been greatly reduced through the elimination of cambial activity. The loss of cambial activity, and the reduction of the development of pericycle and endodermis, as well as the absence of xylem fibers are interpreted here as degrees of advancement toward simplification from the related genus Piper.

The results obtained from studies of the numbers of carpels and chromosomes do not support Yuncker's grouping of the species into subgenera.

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CHAPTER 1.

INTRODUCTION

The genus Peperomia belongs to the family Piperaceae. The precise position of the family Piperaceae with respect to other families of Angiosperms has long been in question (Yuncker, 1958). Bessey (1915) placed this family under the order Ranales. However, Melchior (in Engler's Syllabus, 1964), Cronquist (1957), and Hutchinson (1959) suggested that the family Piperaceae together with Chloranthaceae, and Saururaceae form a distinct group namely the order Piperales. The classification is now generally accepted. Thorne (1964) has proposed that the order Piperales, together with Magnoliales, Laurales, and Aristolochiales should be incorporated with the order Annonales.

Phylogenetically, the family Piperaceae is of uncertain origin. Hutchinson (1959) believes that the family was derived from a ranalian stock, which is considered to be one of the primitive dicotyledonous groups. Balfour (1959) suggests that Piperaceae forms a distinct group, not closely related to other dicotyledons.

Many botanists have made detailed studies of the flora of the Hawaiian Islands (Degener, 1930 and later; Fosberg, 1948; Hillebrand, 1888; Rock, 1913). Approximately 95 percent of the species and varieties of Angiosperms in the present flora are endemic. In the family Piperaceae, only the genus Peperomia is native to the Hawaiian Islands. The genus is represented by 45 described

species (St. John, 1943; Yuncker, 1933, 1938) of which 43 are endemic to the Hawaiian Islands. The majority of the species are distributed on more than one island, though a number of species have very restricted distributions. The geographic distribution of the localized species compared to the widespread occurrence of the other species needs to be examined more closely.

There are approximately 1500 species of the genus Peperomia known, with a distribution throughout the tropical and subtropical areas of the world. Trelease and Yuncker (1950) believed that with additional materials and new evidence for comparison, the number of the known species would possible be reduced, though some new species might be described.

The relationships among the species within the genus present many problems. In grouping the species into subgenera, Dahlstedt (1900) emphasized the importance of fruit structure, and suggested that the genus might be classified into nine subgenera. According to Trelease (1930) some of the subgenera might deserve generic recognition. Unfortunately, the morphological characters of many species are not known in sufficient detail for Dahlstedt's (1930) key to be used in comprehensive classification (Trelease, 1930; Skottsberg, 1947). Studies of comparative morphology, cytology, genetics, cytogenetics, and geographical distribution yielding characters which have proved to be of value in determining phylogenetic relationships in other genera (Babcock, 1947; Gunderson, 1956), have not been carried out for most species.

of Peperomia. There is little information which characters should be considered primitive within the genus, and little is known of the phyletic relationships among the species.

Neither the genus Peperomia nor any other genera of the family Piperaceae have been studied biosystematically. Only a limited number of characters, which often show remarkable variability, have been used for the delimitation of the subgenera. The absence of even vestigial evidence of calyx and corolla raises the question whether the naked condition is a primitive or advanced feature of the floral structure in the family. Whether or not chromosomal biotypes are related to the ecological conditions in which the plants grow is also in question.

The problems which have been outlined above are only a few of those which are presented to botanists studying the family Piperaceae. The need for thorough investigations through the study of anatomy, cytology, genetics, and ecology of its genera and species is emphasized, because such studies may contribute a broader view to the present knowledge and interpretation of the family.

With these problems in mind, four different subjects were undertaken in studying the genus Peperomia:

1. Carpel Morphology:

Johnson (1914) and van Tieghem (1918) suggested that the gynoecium in Peperomia is monocarpellary because only one unilocular ovary was found.

However, Murty (1958), after studying the vascularization and stigma number of the carpels, suggested that the gynoecium of Peperomia was bicarpellary and derived from a tricarpellary condition. In addition to variation in the number of stigmas, the position of the stigma on the carpel can be either apical or subapical (Yuncker, 1933).

The variation in stigma position and vascular supply of the carpel was investigated. The number of vascular bundles in the carpel was correlated with the position of the stigmas as well as the number of the stigmas in each carpel. This study provided evidence that a true monocarpellary condition was found within the genus and also suggested an interpretation of the evolution of stigma position on the carpel.

2. Chromosomes:

The importance of cytological studies in tracing the relationships between the different species of a genus has been demonstrated in other genera (Babcock, 1947; Fernandes, 1951). No detailed cytological work has been carried out in the species of Peperomia which are endemic to the Hawaiian Islands. The chromosome number in only five of the 45 Hawaiian species has previously been reported (Skottsberg, 1959). Despite the large number of the species in the genus, there is little cytological data available for the genus as a whole. Therefore, it was necessary to determine the basic chromosome number of the Hawaiian Peperomia species so that the possible speciation of the genus in Hawaii could be considered.

3. Leaf Arrangement:

In Peperomia the division of the subgenus Sphaerocarpidium into sections is based on the arrangement of the leaves on the stem (Yuncker, 1933). Both opposite and whorled arrangements can be found on the same plant. Murty (1960), on the basis of the anatomical evidence, has shown that the opposite and whorled arrangements of leaves are actually subopposite and pseudo-verticillate and can be derived from an original spiral arrangement.

The arrangement of the leaves at the shoot apex has been studied by dissecting out the apex and comparing the phyllotaxy at the apex with that of the mature region. The study was undertaken to ascertain whether any leaves were whorled at initiation. It was then decided to test whether there was a correlation between leaf arrangement and carpel type.

4. Stem and Leaf Anatomy:

Previous studies on the Hawaiian species of Peperomia have been confined largely to external morphology (de Candolle, 1913; Hillebrand, 1888; Yuncker, 1933). The species in Hawaii are grouped into three subgenera. Two of the subgenera, Micropiper and Sphaerocarpidium, were first described in Dahlstedt's classification (1900), and the third subgenus, Hawaiiana, was proposed by Yuncker (1933). The separation of these three subgenera was based in part on the number and position of the stigmas. Yuncker (1933) pointed out that variation in the appearance of the stigma in the fruiting stage was sufficient to create problems in placing the species in the correct

subgenus, since sometimes stigmas remained undeveloped, and at other times fell off prematurely.

The present study was, therefore, undertaken with the intention of evaluating the trends of specialization within the genus using comparisons of several anatomical characters. It was thought that a new approach, studying both vegetative and reproductive structures, might lead to a better evaluation of taxonomically useful characters. The Hawaiian Peperomia present ideal material for such a study.

The present report deals with the results obtained from only the Hawaiian species of Peperomia. Because of limitation of time and of available materials, 27 Hawaiian species of Peperomia were studied with the view to determining the relationships between these species which may, in turn, be useful as a basis for further study in other species of the genus. To validate the suggestions outlined in this paper larger samples of other species collected from different parts of the world would be necessary.

CHAPTER 2.

HISTORICAL REVIEW

Taxonomy:

The family Piperaceae contains 11 genera (Trelease, 1930): Photomorphe, Manekia, Sarchorachis, Trianaeopiper, Lindeniopiper, Arctottonia, Ottonia, Piper, Verhaullia, Piperanthus, and Peperomia. The genus Peperomia was first described by Ruiz and Pavon (1798) and includes many of the species which were initially classified as members of the genus Piper by Linnaeus (1753). In his classification of the family Piperaceae, Miquel (1843) recognized seven subgenera of the genus Peperomia, namely Tildenia, Rhynchophorum, Micropiper, Panicularia, Phyllobryon, Acrocarpidium, and Erasmia. He based his classification primarily on the fruit structure, but included some species from specimens without fruits, using theig vegetative characters. Dahlstedt (1900) considered these characters to be too variable to be used as diagnostic attributes. De Candolle (1869), in a monograph on this genus, did not recognize subgeneric groups. However, Henschen (1873) divided the genus into two main groups, Acrocarpidium and Eupeperomia. The latter was subdivided into two subgroups, Micropiper and Rhynchophorum. Using characters exhibited by mature fruits, Dahlstedt (1900) subdivided the genus into nine subgenera: Acrocarpidium, Ogmocarpidium, Erasmia, Pleurocarpidium, Tildenia, Panicularia, Rhynchophorum, Sphaerocarpidium, and Micropiper. Though most of these names are identical with those of Miquel (1843), the

characters used in the delimitation of the subgenera were somewhat different. In his studies on the geographic distribution of American peppers, Trelease (1930) added two more new subgenera, Hemirhynchophorum and Leptorhyncum, which were segregated from Tildenia and Rhynchophorum, respectively, of Dahlstedt (1930). In grouping the species into subgenera, Dahlstedt's (1900) classification has been generally followed (Skottsberg, 1957; Trelease, 1930; Yuncker, 1933). All previous classification into subgenera have included relatively few of the 1500 described species of Peperomia; the validity of such groupings is questionable.

Hooker and Arnott (1832) described 2 species of Peperomia, P. membranacea and P. leptostachya, from the Hawaiian Islands. These were the first species of the genus described from Hawaii. Miquel (1843, 1844) included descriptions of 6 new Hawaiian species in his monographs on the Piperaceae. In addition to these, Wawra (1869) described two more species. Hillebrand (1888) recognized 19 species of Peperomia in his Flora of the Hawaiian Islands. However, he doubted the validity of some of his specific names due to lack of comparison with the materials from the other herbaria. De Candolle (1913) in a revision on the Hawaiian species of Peperomia recognized 73 species, of which 67 were endemic. No grouping of the species into subgenera was included in any of the above mentioned papers. After studying most of the type specimens, Yuncker (1933) revised the Hawaiian species and recognized only 38 species, which included 8 new species. To this number 7 further species were added (St. John,

1943; Yuncker, 1937, 1949). Yuncker (1933) made an attempt to group the species into their corresponding subgenera, following Dahlstedt's (1900) classification. Accordingly, one of the species fell into the subgenus Micropiper, 20 species into the subgenus Sphaerocarpidium, and the remaining 17 species into the new subgenus Hawaiiana (Yuncker, 1933). The subgenus Sphaerocarpidium was further divided into sections Alternifoliae and Verticillatae on the basis of leaf arrangement (Dahlstedt, 1900). According to Trelease (1930) all Hawaiian species would fall into the subgenus Sphaerocarpidium, unless a new section was created which would include the species with a bilobation of the stigma. These species were grouped into a new subgenus Hawaiiana, by Yuncker (1933); he believed that the species which were included in this subgenus were all endemic to the Hawaiian Islands. Because the interrelationships of species are not known, and because of the variability of the characters used to separate them, the validity of the subgenera is uncertain. However, it may be practical and convenient for some purposes to continue to recognize them.

Distribution:

The genus Peperomia has a world-wide tropical and subtropical distribution. However, only the distribution of the subgenera of the genus Peperomia found in America has been reported here (Dahlstedt, 1900; Trelease, 1930). The fruit characters of most species from Africa (Baker and Wright, 1913), India

(Miquel, 1846), and the Philippines (Quisumbing, 1930) have not been described adequately. As a result, the grouping of these species into their corresponding subgenera has not been done yet.

From the study of the geographic distribution of its species, Trelease (1930) concluded that Peperomia may be considered of American origin, because the species found in America are more numerous and more segregated into major types than those from other parts of the world.

Comparing the Hawaiian species of the subgenus Sphaerocarpidium with both the Philippine species which were thought to represent the Indo-Malayan species and the American species of the same subgenus, Yuncker (1933) concluded that the Hawaiian species were more closely allied to the American species than to the Indo-Malayan ones. However, Skottsberg (1946), in his discussion of the possible affinities of the species in Juan Fernandez with those of Hawaii and Indo-Malaya, suggested that the species included in the subgenus Hawaiiana should be linked with the Indo-Malayan species. Fosberg (1948) also pointed out that the flora of the Hawaiian Islands was largely derived from the Indo-Malayan, although there is also a strong American element. He suggested that there were three immigrants of the genus Peperomia into the Hawaiian Islands; one was of Indo-Pacific, one of American, and another of uncertain origin.

The distribution of the species of Peperomia in Hawaii is presented in Table 1.

It can be seen from Table 1 that Maui and Molokai have more species than the other major islands. Yuncker (1933) suggested that both Molokai and Maui present habitats more favorable for the growth and the development of Peperomia. However, no explanation was offered to substantiate this comment, and it would not seem to be a valid hypothesis when one considers the climate and topography of each of the islands.

CHAPTER 3

MATERIALS AND METHODS

Fresh specimens collected in the field were used in all the investigations reported in this thesis. Samples from 242 collections of 26 species of Hawaiian Peperomia were included. Those were collected from various places on the five major Hawaiian Islands.

The species used in anatomical studies have been arranged in alphabetical order (Table 2); also included is the location of collections and collectors. Undetermined specimens are included at the end of the list and the closest affinity is indicated. Identification was based on the papers by Yuncker (1933) and Degener (1943), and confirmed by examination of specimens in the Herbarium of B. P. Bishop Museum, Honolulu, Hawaii. Voucher specimens have been placed in the Herbarium Bogoriense of the National Biological Institute of Indonesia.

The unpredictable periods of flowering of members of this genus made it difficult to secure flowering material of all species. All specimens listed (Table 2) have been examined for details of leaf and stem anatomy. An asterisk indicates that fruiting material has been studied; samples for which chromosome counts of pollen mother cells have been made are marked with a plus sign.

Stem samples were cut from the region close to the basal part of an erect stem of each plant. Mature leaves were used in this study and only the median

parts were taken for transverse sections. Observations recorded are based on such samples collected from three plants from each collection. As well as comparing similar characters of different species, these features in the same species from different localities were also compared. Paradermal sections from the median parts of mature leaves were made to study stomata. The position of stomata with respect to the epidermal cells was shown by photographing them and then tracing them on paper. This method was used because details of unstained sections did not show up clearly in the photographs.

For studies of the trichomes, leaves were cleared as follows: The leaves were boiled in water for three to five minutes, then transferred to boiling 70% alcohol for an additional three to five minutes. This procedure was followed by clearing in 70% lactic acid maintained at 60° C for several weeks. After the materials were cleared, the lactic acid was replaced by 70% glycerin and kept at room temperature for 24 hours. Then the materials were transferred to and kept in 50% glycerin for 24 hours. The time for clearing could be made shorter by using chloral hydrate after 70% alcohol and before 70% lactic acid. Cleared materials were dehydrated in ethyl alcohol and stained in a 1% safranin solution, using a 1% Fast Green solution as a counterstain. The best results were obtained from the modified clearing technique of Bersier and Bocquet (1960). Fresh leaves were immersed in a mixture of: 1 phenol : 1 lactic acid : 2 chloral hydrate at 60° C for three to five days. For thick leaves, one or two changes of solution were necessary. Trichomes were studied without further staining.

Fresh materials for sectioning were killed in FAA for at least 24 hours. The materials then were dehydrated, using a tertiary butyl alcohol series. This procedure was followed by infiltration with Parowax and embedding in Tissuemat according to a standard paraffin embedding technique (Johansen, 1940). After materials were embedded, sections were cut at 8 to 12μ; overstained with 1% safranin, and counterstained with 1% Fast Green. The optimum time in Fast Green was four to six minutes, after which time the pit areas of parenchyma cells were visible. For preliminary studies, transverse sections of stems and leaves were cut by hand. Tests using Sudan IV, Phloroglucinol-HCl, Ruthenium red, and Aniline blue were also conducted to show the presence of oily substances, lignified cell walls, pectic substances, and cellulose.

Leaf arrangement was studied by dissecting the shoot apices under a dissecting microscope; this was followed by a comparison with leaf arrangement in fully developed plants. Serial transverse sections of shoot apices of P. hypoleuca were made to trace the course of the bundles.

For the study of chromosomes during meiosis, stamens were fixed in Farmer's solution (3 parts absolute alcohol plus 1 part glacial acetic acid); this solution gave satisfactory results. "Squashes" of the stamens were made permanent by the dry ice method (Conger and Fairchild, 1953).

Fruits in several stages of development, as well as the whole spike, were cleared in chloral hydrate and stained in Phloroglucinol-HCl. Such material

was mounted in 20% glycerin. After carefully sealing with nail polish, the slides were reasonably permanent. For detailed study of the mature fruits, several sections of the inflorescence with mature fruits were made using paraffin methods. Drawings of the trichome distribution and fruit vascularization were made by the projection method described by Saylor (1961). Photomicrographs were taken with a Wild Microscope, on Panatomic X film developed for 8 minutes in Microdol X.

CHAPTER 4.

GENERAL MORPHOLOGY

Peperomia species are herbs, from a few centimeter to a meter tall. The Hawaiian species are commonly found in moist habitats. P. reflexa and P. leptostachya favor drier habitats; they are usually found on rocks or in crevices of rocks. P. alternifolia, P. cookiana, P. dextrolaeva, P. hypoleuca, P. latifolia, P. membrancea, and P. oahuensis are not uncommonly epiphytic. Of the Hawaiian species, P. subpetiolata, P. hirtipetiola, and P. macraeana are among the largest with heights of $\pm$ 1 m, and diameters of $\pm$ 2.0 cm at the base of the stems, whereas P. waikamoiana is the smallest with a height of $\pm$ 6 cm, and a diameter of $\pm$ 2 mm. The roots are of the fibrous type, the production of which is never luxuriant. Most of the Hawaiian species produce stoloniferous stems allowing adventitious roots to develop, especially in the nodal regions. The cross sections of the stems are circular. Zigzag stems are found in the species having alternate leaf arrangement, i.e., P. alternifolia, P. dextrolaeva, P. oahuensis, and some samples of P. hypoleuca. The leaf arrangement of the Hawaiian species is alternate, opposite, or whorled. The size, and the shape of leaves varies greatly (Trelease and Yuncker, 1950; Quisumbing, 1930). A large number of species are more or less pubescent, with trichomes showing a considerable range in size. Glandular-like secretory cells are present in

leaves and sometimes in the other parts of the plants. The leaves in the majority of species have palmate venation. The spikes are terminal, axillary or leaf opposed. Of the species found in the Hawaiian Islands, P. relfexa produces terminal spikes only, whereas P. alternifolia, P. dextrolaeva, and P. oahuensis produce axillary spikes only. The rest of the Hawaiian species have both axillary and terminal spikes, either solitary or clustered. Among the species having the longest spikes are P. latifolia, P. oahuensis, P. remyi, P. sandiwicensis, and P. subpetiolata, i.e., $\pm$ 15 cm long. The shortest spikes, $\pm$ 8 mm long, found in P. waikamoiana. P. sandwicensis is characteristic in having the longest peduncle. The flowers are sessile, perfect, and subtended by bracts. The stamens are two in number, laterally attached at the base of the ovary and early deciduous. The fruit is one seeded and of a drupe type. The surface of the fruit is covered with viscid exudates, allowing the fruit to attach to any object which comes in contact with it. Although no evidence could be given, Yuncker (1933) believed that the fruits are disseminated by birds. A considerable number of species produce a persistent pseudopedicel which develops from the rachis, elevating the fruits at maturity. The stigmas are smooth or penicillate, single, two, or three.

Attempts to germinate seeds were made by putting mature fruits on wet filter paper in petri dishes. Although seedlings were obtained, most of the fruits rotted after several days due to fungal growth around the fruits. Some of the Hawaiian species such as P. sandwicensis, P. leptostachya, etc. could

be propagated through leaf cuttings. This is done by placing the leaves on wet vermiculite and covering them with a piece of glass; the box was kept in an air conditioned room.

CHAPTER 5.

FLOWER MORPHOLOGY

Introduction:

The flowers of Peperomia are minute and arranged spirally on a spike inflorescence. Each flower lacks a perianth, but it is subtended by a bract. The stamens with bilocular anthers arise from the axis near the base of the ovary. Such a condition is quite unusual among Angiosperms (Johnson, 1914).

In reviewing the use of floral anatomy in the solution of morphological problems, Puri (1951) stated that, anatomically, the carpel is the most complex of all floral organs because it is the last organ formed on the floral axis. De Candolle (1827) regarded a carpel as a leaf-like organ, folded upward. From the studies of vascular anatomy, Arber (1937), Eames (1961), Sprotte (1940), and Troll (1934) have supported this conception. Because it is a leaf-like organ, Eames (1961), and Sinnott and Baily (1914) have suggested that the primitive carpel, like the primitive leaf, is a three-trace appendage. Melville (1962) rejected this theory, however, because of its failure to explain the inconsistencies in carpel morphology.

In the genus Peperomia, there is no agreement as yet as to how many carpels compose the gynoecium. Van Tieghem (1891) considered the gynoecium of Peperomia as unicarpellate. This condition was confirmed by Johnson (1914) in P. hispidula. Although developmental studies have not shown the coalesence of

three carpels, Johnson (1914) assumed that the carpel of Peperomia was derived from a hypothetically more primitive type characteristic of Piperaceae, i.e., three carpels with six vascular bundles in the wall of the ovary, through a reduction from nine or some other original number.

Eckardt (1937) discussed the two probable lines of evolution which gave rise to the carpel of Peperomia. In the first line, the tricarpellary condition of Piper was a prototype of the carpel of Peperomia, whereas, in the second line, the carpel of Peperomia has evolved quite independently from the Piper type. Murty (1958) agreed with the view that the carpel of Peperomia is related to those of Piper and Photomorphe. His conclusion was based on the presence of more than one stigma in the carpel. He then interpreted the ovary of Peperomia as being pseudomonomerous and bicarpellary.

The ovule of Peperomia is single, erect, and apparently basal in position. Sachs (1875), and Worsdell (1904), believed that in this condition the ovule represents a direct continuation of the floral axis. However, Eames and MacDaniels (1947) believed that no angiosperm ovule is cauline; in other words, the ovule is foliar in origin. Murty (1958), with the aid of anatomical evidence, interpreted the ovule of Peperomia as subbasal and derived from a lateral position. Bechtel (1921), Benson and Welsford (1909), and Le Roy (1955) gave similar interpretations for Boehmeria, Myrica, and Juglans, respectively. In recent years, anatomical and paleobotanical evidence has suggested that the foliar theory of the origin of the angiospermous ovule, carpel, and stamen is

questionable (Meeuse, 1966). Through anatomical studies Barnard (1958) has demonstrated what he believes to be the cauline nature of Juncus and Bulbine ovules. Meeuse (1966) and Moeliono (1962) suggested that the ovule is axial in origin in both Engelhardia and Stellaria. Meeuse's (1966) explanation of ovule phylogeny is based upon palaeobotanical studies, and may offer a solution for many problems in angiosperm morphology. However, these theories need further substantiation before they can be generally accepted in place of the theories of foliar origin of carpels, which seem to have the weight of the evidence behind them.

Results and Discussion:

Teratological inflorescences were observed in many species found in the Hawaiian Islands and Southern Polynesia (Yuncker, 1927). Among the Hawaiian species with abnormal flowers, P. hirtipetiola, P. reflexa, and P. sandwicensis have more than one type of abnormal development. In the present study, two more species from the Hawaiian Islands were found to have abnormal inflorescences; these are P. membranacea and P. leptostachya. In those two species, the spikes branch near the tip; leaves were found on the inflorescence axis just above the point of branching.

The most comprehensive anatomical work on the flowers of Peperomia species was that of Murty (1958) who examined twelve species from all over the world. In the present study, flowers of 27 species of Hawaiian Peperomia were investigated (Table 3).

Trichomes similar to those of the leaves were found on the peduncles of all species observed except P. alternifolia, P. dextrolaeva, P. globulanthera, P. hawaiiensis, P. hesperomanii, P. koolauana, P. membranacea, and P. oahuensis. Trichomes were also found on the rachis of P. reflexa. Glandular hairs, oil cells, and stomata were found on the peduncle and inflorescence axes of all species observed.

The occurrence of either single or divided stigmas has been noted in Hawaiian Peperomia species (Yuncker, 1933). Among the abnormalities observed in the genus Peperomia, he listed the presence of three stigmas in P. sandwicensis and P. eekana. It is shown in Table 2 that three stigmas occur commonly in P. erythroclada, P. hirtipetiola, P. kalihiana, P. latifolia, and P. lilifolia (Plate 1A). No styles were observed in any of the species studied, except in P. reflexa where a short style was observed on a fully developed carpel. The number of stigmas of all species studied is shown in Table 3.

The peduncle contains six to eight vascular bundles which branch off from the stem bundles. The vascular bundles are not arranged in a ring, but are scattered similarly to those in the stem. Upon entering the rachis, such bundles branch and give rise to flower traces (Plate 1B).

Although Rousseau (1927) presented an illustration of a Peperomia inflorescence showing that the flower traces branch off from the main axis at a different level from the bract trace, the pattern of vascularization in

all of the Hawaiian species studied, except some flowers of P. leptostachya (no. 175), agrees with that described by Murty (1958). Each flower and its corresponding bract receives a single vascular strand which branches from a main vascular bundle (Plate 1B), and passes obliquely upward through the parenchymatous cells on the rachis. This vascular bundle then splits into two, one bundle going to the flower and the other to the bract. In some flowers of P. lilifolia (sample 195), the flower trace and the bundle of the bract diverge from the vascular bundle of the axis separately (Plate 1E). Murty (1958) reported the occurrence of a single bundle supplying two flowers in P. magnoliaefolia. A similar condition was found in the present study in P. oahuensis (sample 169), and P. lilifolia (sample 172) (Plate 1D).

Two lateral branches are given off by the floral trace at the base of the carpel. These bundles are the stamen traces (Plate 1B). Each of the stamen traces ends up in a plate of tracheids, as is demonstrated in P. lilifolia (sample 179) (Plate 1C).

Carpel:

On entering the carpel, the floral vascular tissue divides into two or more unequal branches. In P. cookiana, P. dextrolaeva, P. erythroclada, P. helleri, P. koolauana, P. leptostachya, P. membranacea, P. remyi, P. waikamoiana, there are two unequal bundles in the carpel. In cases where there is only one stigma, as in P. cookiana, P. koolauana, P. leptostachya, P. membranacea, P. remyi, P. reflexa, P. waikamoiana, the abaxial bundle, which is the larger, passes upward through the ovary wall and forms a ring at the base of the stigma

(Plate 2A). In older fruits, the ring develops into a mass of tracheids (Plate 2B) which possibly is similar to that described by Murty (1959) in P. magnoliaefolia. In some flowers of P. 170 the adaxial bundle is larger than the other bundle. P. alternifolia, P. dextrolaeva, P. ellipticibacca, P. helleri, P. hesperomannii, P. kalihiana, P. latifolia, P. oahuensis, and P. sandwicensis have flowers with both one and two stigmas in their inflorescences. In these species, both single and double vascular bundles are found in the carpels. Some of the carpels which have two stigmas possess only one vascular bundle in the ovary wall. In this case, in addition to the formation of a ring at the base of the stigma, there is a protrusion of tracheids from the ring toward each stigma (Plate 2C). These protrusions end blindly at the base of the stigma. Skottsberg (1947) observed double stigmas and two vascular bundles in the ovary wall of P. barteroana and P. tristanensis. The abaxial bundle was larger than the adaxial one in these species. Murty (1959) noticed a similar condition in P. fraseri. Among the Hawaiian species with double stigmas, P. eekana (sample 132), P. kalihiana (sample 27), P. erythroclada (sample 235), P. subpetiolata (sample 244) and P. lilifolia (sample 100) have one, two, or three vascular bundles in their ovary walls, while P. latifolia collected from high elevations (Castle Trail and Konahuanui) has two and three vascular bundles. In these seven preceding species the occurrence of three stigmas was also observed. Although Skottsberg (1947) reported that the abaxial bundle was larger than the adaxial one in the species which he observed, in the Hawaiian species the bundles are approximately the

same size. It is interesting to note that the ovule trace is associated with the adaxial bundle (Plate 2D). The number of vascular bundles per ovary with the corresponding number of stigmas is presented in Table 4.

A pseudopedicel (Plate 2E) develops as an outgrowth of the rachis in all the specimens studied in P. erythroclada, P. sandwicensis, and P. waikamoiana. This dome-like structure does not develop until the fruit reaches maturity. It consists of parenchymatous cells, with elongate cells at the periphery. As a consequence of rapid division and elongation of the parenchymatous cells of the cortex of the rachis in the formation of the pseudopedicel, the vascular bundle to the bract appears bent (Plate 2E).

Ovule:

The genus Peperomia has a single, orthrotropous ovule, with a single integument, in each carpel. However, Fisher (1914) reported the presence of a two-lobed ovule in P. verticillata. Of the two lobes, one developed into a seed, and the other remained sterile. Similar conditions were found in P. reflexa, P. cniapas, and P. sp3., according to Murty (1958). In all of the Hawaiian species studied, no bilobation of the ovule has been observed. Johnson (1914) reported the shrivelling of the ovules in P. hispidula, which according to him is probably due to the non-fertilization of the egg in the ovule. This condition is found in P. oahuensis (sample 169 and 171).

The ovule trace is single, and considered to be either axial (Johnson, 1914; Worsdell, 1904) or lateral (Murty, 1958) in position. In P. hispidula, the

bundle ends with bent and spiral tracheids (Johnson, 1914). This condition is observed in most of the Hawaiian species of Peperomia. Murty (1958) observed the curving of the ovule trace toward the abaxial side of the ovary and used this evidence to interpret the ovule as lateral. In the present study, the curvature of ovule traces was clearly shown in P. lilifolia and P. cookiana. Some fruits of P. lilifolia (sample 172) have an extra protrusion where the vascular bundle starts to curve. Straight ovular traces are commonly found in Hawaiian species (Plate 2D).

The present study has revealed the occurrence of one, two, or three stigmas per gynoecium. The number of vascular bundles in the carpel is not necessarily the same as the number of the stigmas present. The following conditions have been found in the Hawaiian species:

a. single stigma with single vascular bundle.

b. two stigmas with one or two vascular bundles.

c. three stigmas with two or three vascular bundles.

There are different opinions concerning the number of carpels in the Peperomia gynoecium. Van Tieghem (1891, 1918) and Johnson (1914) considered Peperomia to be unicarpellate. Eckardt (1937) discussed two ways of interpreting the number of the carpels in Peperomia. According to the first interpretation the Peperomia gynoecium has been derived from a tricarpellary Piper type, whereas in the second interpretation, the carpel of Peperomia has evolved independently from those of Piper type. Murty (1954) interpreted the

carpel of Peperomia as being pseudomonomerous and bicarpellary, but suggested possible past connections with types such as Piper and Pothomorphe.

P. lilifolia, which is classified in the subgenus Hawaiiana (Yuncker, 1933), is typical of the two stigmatic condition. In the present study, it was found that the majority of flowers had two stigmas accompanied by two carpellary bundles. However, flowers with three stigmas and three carpellary bundles were not uncommon. This condition was also common in P. erythroclada, P. hirtipetiola, and P. kalihiana. P. latifolia of the subgenus Sphaerocarpidium which were collected from high elevations has either one, two, or three vascular bundles. Yuncker (1927) found fruits with three stigmas in P. eekana and P. sandwicensis, but described them as teratological. The three carpellary bundles do not fuse at one common point. The first two bundles join at one level, whereas the last bundle joins one of the two at a higher level. It was observed that the ovule trace was always associated with the uppermost carpellary bundle. According to Eames (1961), most solitary, basal ovules have traces from the ventral bundle. Taking this into account, the ovule trace of Peperomia is considered to be a ventral bundle. Thus, the three carpellary bundles observed in the ovary wall are interpreted here as the dorsal bundles. This interpretation is similar to that presented for the vascularization of Sarcandra (Swamy and Bailey, 1950).

P. latifolia of the subgenus Sphaerocarpidium (Yuncker, 1933) represents a species having both one and two stigmas in its inflorescences. The number

of dorsal carpellary bundles is either one or two, except in those samples collected from high elevation. Flowers with two stigmatic regions have either one or two vascular bundles in their ovary walls, whereas the single-stigma flowers have only one. The ovular or ventral trace is either straight or bent. Sometimes in the region where the tracheary elements start to bend, a projection of tracheary elements is found. In P. sandwicensis of the same subgenus, the pattern of carpel vascularization is slightly different. Here, either one or two stigmas may be found, but there is always one dorsal bundle.

P. membranacea is an example of species having one stigmatic region on the abaxial side and a conspicuous protuberance on the adaxial side of the ovary (Plate 2F). Murty (1959) considered this protuberance as a non-receptive stigma which sometimes becomes receptive, thus forming the second stigmatic region. He gave two bits of evidence to support this interpretation, i.e., the presence of a vascular bundle in the protuberance, as well as the occassional presence of a second receptive stigma. Studies of the Hawaiian species considered in this thesis do not support such interpretation. Plate 2F demonstrates a mature fruit in which the protuberance has developed beside the stigma proper, whereas Plate 2A shows a younger fruit in which the protuberance has not developed yet. Note the presence of only a single dorsal carpellary bundle in the ovary wall. Just below the stigma the dorsal carpellary bundle forms a loop. If this fruit is sectioned longitudinally,

perhaps only a part of the loop is included in the section, thus giving an impression that the dorsal carpellary bundle is extended into the protuberance as outlined by Murty (1953). Therefore, the protuberance is not a sterile stigma but merely a protuberance of the ovary wall.

The Hawaiian species show a gradation of the stigma number as well as of dorsal carpellary bundle number. In syncarpous gynoecia, the carpel number has been reduced from an ancestral many to few to one (Eames, 1961). The reduction of the carpel number from three to two to one is well portrayed. in the Hawaiian Peperomia species. This is shown through the reduction of the number of stigmatic regions, as well as the number of the dorsal bundles. From the joining of the dorsal bundles, it is concluded that the fusion of the carpels to each other has occurred at different levels. This condition was also found in spirally arranged carpels of Annonales, Eupomatiaceae, Zygogynum, and some Berberidaceae (Eames, 1961). It is suggested here that the species with one stigma and one dorsal carpellary bundle represent a true monomerous unicaprellate ovary as mentioned by Johnson (1914). The Hawaiian species, therefore, provide evidence that the primitive condition in the genus may have been tricarpellary, and that the present day species show all stages in the reduction to two carpels and finally to one carpel.

There is no agreement as yet on the nature of the ovular trace of Peperomia. Worsdell (1904) considered the single ovule of Peperomia to be an axial

structure. Murty (1958), however, interpreted the bending of the ovular trace to be an indication for the lateral nature of the ovule. Both bent and straight ovular traces are commonly found in the carpels of the Hawaiian species. Further, a projection of tracheary elements was found in the region where the ovular bundle starts to bend. This tracheary projection was only found in carpels having one or two dorsal carpellary bundles, but not in those with three bundles in the ovary walls. Thus, it is possible to interpret the projection as a vestigial bundle representing the third and the second dorsal carpellary bundles. Therefore, the bending of the ventral or ovular bundle alone cannot be used to interpret the lateral position of the ovule. The occurrence of the projection in the ventral or ovular trace suggests that the first dorsal bundle to disappear was the uppermost one. This was followed by the next upper bundle. In the flowers with a single dorsal carpellary bundle, the ovular trace is greatly reduced in length.

CHAPTER 6.

CHROMOSOME STUDY

Introduction:

In selecting characters for taxonomic purposes, Darlington (1956), Stebbins (1959), and Love (1960) regarded cytological characters as the most important. However, Babcock (1947) stressed the importance of not using chromosome data alone for taxonomic purposes, unless these are related to other morphological evidence. That the chromosomal characters in Primula are as variable as other characters was shown by Smith (1913). Davis and Heywood (1963) distinguished between the value of cytological evidence for comparative taxonomic purposes, and for making evolutionary interpretations. For comparative taxonomic purposes, the cytological characters are not necessarily considered important, because the visible cytological characters are not always directly concerned with heredity mechanisms. However, for interpreting the phylogenetic relationships between species, cytological evidence, combined with geographical distribution, is often of the greatest importance.

In the family Piperaceae, the genus Piper is considered to be taxonomically related to the genus Peperomia (Johnson, 1914; Murty, 1959; Sinnott, 1906). The homogeneity in numbers and shapes of chromosomes has been shown in both genera (Brown, 1908; Hauser, 1906; Mathew, 1960). Sharma and Bhattacharyya

(1959) provided cytological confirmation for the view that the genera are related. According to these authors, all the species of Peperomia investigated were considered to belong to a single evolutionary series. By comparing the genome of Peperomia species, the genus can be grouped into two categories: one group having multiples of n = 11, the other of n = 12. Sharma and Bhattacharyya (1959) suggested that there is some indication that n = 12 was derived from n = 11, through the duplication of some members of the complement. The somatic chromosome number of the Hawaiian Peperomia species, as reported by Skottsberg (1953) are: P. eekana, 2n = 24; P. erythroclada, 2n = 28; P. hawaiensis, 2n = 42 to 46; P. hesperomannii, 2n = 48, 66; and P. lilifolia, 2n = 42.

Variations in the chromosome complement of somatic tissue in the same individual of some species of Peperomia have been reported (Sharma and Chattacharyya, 1959; Blot, 1960). Species differing in karyotypes have been recorded for several genera (e.g., Babcock, 1947; Gates, 1901; Sharma and Bhattacharyya, 1959; Stebbins, 1951). In case more than one chromosome number were recorded, the basic chromosome set was estimated from the number with the highest frequency. Sharma and Sharma (1956), Sharma and Bal (1956), and Sharma and Mazumdar (1956) have shown that a variation in the chromosome complement is of common occurrence in all the vegetatively-propagated plants which were investigated. In the genus Peperomia, though most of the species produce flowers, the main method of propagation in

horticulture is by vegetative means. However, one can expect somatic variations to occur in nature but such variations, particularly in chromosome number, would normally be eliminated during meiosis. Chromosome irregularities, including "lagging" and precocious separation, have been observed during meiotic division (Sharma and Bhattacharyya, 1959; Blot, 1960). These meiotic disturbances resulted in a high percentage of sterile pollen grains. There has been no explanation for the cause of these irregularities. Meiotic chromosome abnormalities, with consequent pollen sterility, have not been reported so far for the Hawaiian species.

Results and Discussion:

It has been mentioned that the inflorescence of the genus Peperomia is a spike, with the flowers maturing from the base to the apex. This condition often permits one to find different stages of meiotic divisions in a single inflorescence, although in most cases meiotic divisions occur very early in the development of the inflorescence.

The duration of the different stages of meiotic division appeared to be quite variable. Metaphase I, Anaphase I, Metaphase II, and Anaphase II occurred very rapidly. In comparison to the great number of anthers formed in the inflorescence only a very few anthers showed these stages. On the contrary, Prophase I, Telophase I, Prophase II, and Telophase II were abundant in number.

The basic chromosome number of the genus Peperomia is x = 8, 11, 12 (Darlington, 1945). Tischler (1956) reported the number as x = 11 and 12 only.

The latter finding was confirmed by Sharma and Bhattacharyya (1959) and Blot (1960).

In the present study, 20 species of Hawaiian Peperomia were investigated for chromosome numbers and for meiotic irregularities. Only the gametic chromosome number is reported here, since attempts to obtain mitotic chromosomes were unsuccessful because of unfavorable greenhouse conditions. The meiotic chromosome counts of the 20 species are presented in Table 4; these counts were made from Metaphase I stages.

The only report on chromosome numbers in Hawaiian Peperomia species is that of Skottsberg (1953). He listed somatic numbers of seven specimens belonging to six species (Table 5).

The gametic chromosome counts in P. ellipticibacca, P. hawaiiensis, P. hesperomanii, and P. lilifolia do not correspond with the somatic counts reported by Skottsberg (1955). Out of the 20 species investigated, five are polyploids, and belong to the same subgenus, i.e., Sphaerocarpidium. The remaining species have the same number of n = 22, except P. 138 and some P. lilifolia, which have n = 24. The presence of satellites was recorded in P. 56.

Meiotic abnormalities were observed in P. argyrea, P. pellucida (Sharma and Bhattacharyya, 1959), and P. resedaeflora (Blot, 1960). These abnormalities included lagging and early separation of the chromosome in P. argyrea and P. pellucida, while in P. resedaeflora there is pollen sterility.

Two bivalents have been observed in P. pellucida (Sharma and Bhattacharyya, 1959), in which n = 24. A trivalent has been observed in P. lilifolia.

Chromosomal irregularities were also observed in some of the Hawaiian species of Peperomia. The major abnormalities observed were:

1. Precocious disjuntion.
2. Unpaired chromosomes at M I.
3. Lagging chromosomes at T I and T II.
4. Bridges with and without associated fragments at T I and T II.
5. Micronuclei in the cells of tetrads.
6. Abnormal contraction of chromosomes.

All stages in Prophase I are too complex to permit a detailed analysis of chromosome pairing (Plate 3B) but little evidence of Prophase irregularities was found.

1. Precocious disjunction and unpaired chromosomes:

Various irregularities in the separation in Anaphase movement of the chromosomes were observed. In addition to the normal disjunction, precocious separation of one, two, or three bivalents was observed (Plate 3D). Sometimes an unpaired chromosome was oriented as if moving toward a pole. Whether the unpaired chromosome was the result of desynapsis or asynapsis was not clear from the observations, since no good diakenesis stages were obtained.

Unpaired chromosomes were observed at M I in P. cookiana, P. leptostachya, P. hypoleuca, P. globulanthera, and P. membranacea. The highest

frequencies of precocious disjunction were found in the same plants in which the highest proportion of unpaired chromosomes occurred at M I (Plate 3C).

The most abnormal meiotic divisions were observed in P. cookiana, in which many phases of the meiotic cycle were drastically disturbed. The unpaired chromosomes were so abundant that it was difficult to find any normal M I figures in which all of the bivalents are aligned on the equatorial plate.

2. Lagging chromosomes:

Cells containing lagging chromosomes at T I were observed in P. cookiana, P. hypoleuca, and P. hawaiiensis. The frequency of such cells, however, was quite low. Lagging chromosomes at T II were not observed. The lagging chromosomes were usually oriented as if moving toward the poles.

3. Bridges:

Chromosome bridges usually unaccompanied by chromosome fragments were observed in P. cookiana, P. ellipticibacca, and P. leptostachya. Most of the bridges recorded were at T I (Plate 3F), although it was possible to identify T II bridges in P. cookiana and P. ellipticibacca.

4. Abnormal contraction of chromosomes:

P. cookiana possesses an abnormal chromosome contraction in M II, combined with a pronounced abnormality in the process of meiosis. In M I there was always an extremely strong contraction and the bivalents have an almost spherical shape. In some cases an extremely strong contraction was

observed in M II as shown in Plate 3E. Three poles were observed in P. waikamoiana (Plate 4A).

Table 6 summarizes the irregularities of meiotic division in some species of Hawaiian Peperomia.

4. Polymitosis:

The irregularities which occurred in early phases of the meiotic cycle led to the formation of micronuclei in the tetrad stage. This abnormality resembles to a great extent the polymitosis in maize described by Beadle (in Johnsonn, 1934). It was not clear whether the tetrad nuclei enter prophase again after a short stage as described in the genus Alopecurus (Johnsonn, 1934). Plate 4B demonstrates a tetrad with polymitotic telophase. The size of the nuclei varies in one microsporangium as shown in P. leptostachya (Plate 4B). Sometimes, still further division takes place in the microspore proper (Plate 4C). As a result, pollen sterility is high (Plate 4D). In P. globulanthera, the shapes of pollen grains also vary. Table 7 summarizes the abnormalities of micronuclei and the percentage of pollen sterility. Fully stained pollen grains are considered as fertile, whereas unstained grains are sterile. Usually the size of the unstained grains is greatly reduced.

The pollen grains are spherical, with a thick exine. There are two types of pollen exine, smooth and rough. P. latifolia has a smooth exine, while the rest of the Hawaiian species studied have rough surfaces.

The species of Peperomia from outside the Hawaiian Islands investigated by previous authors show 2n = 16, 22, 24, 44 chromosomes (Abele, 1923; Brown, 1908; Hauser, 1918; Johnson, 1914; Martinoli, 1948; Sharma and Bhattacharyya, 1959; Sugiura, 1936), with the majority of the species containing 2n = 24 chromosomes. The somatic chromosome number of the six Hawaiian species reported by Skottsberg (1955) are 2n = 28, 36, 42, 46, 48 and 66. Of the 20 species investigated in the present paper, 15 have n = 22, two have n = 24, three have n = 44, one has n = 48, and one has both n = 22, and n = 24. Thus, unlike the non-Hawaiian species which mostly show multiples of 12 chromosomes (Sharma and Bhattacharyya, 1959), the Hawaiian species seem to have the tendency of possessing chromosomes in multiples of 11. Of the 20 species reported here, four species exhibited the doubling of chromosome complements as compared with the rest of the species studied.

Based on their investigation of some Indian species, Sharma and Bhattacharyya (1959) concluded that all species of Peperomia, except the one having 2n = 16, constituted a single evolutionary series, starting with 11 chromosomes in the basic set, which later have given rise to the species with a haploid complement of 12 chromosome through duplication of some members of the complement. Although a large number of species constitute this genus, cytological data have been reported from only few species. Therefore, the Hawaiian species can be used to provide additional evidence in considering the possible evolution of the genus Peperomia.

The occurrence of chromosomal biotypes which are related to ecological conditions has been shown in P. pellucida (Sharma and Bhattacharyya, 1959). In this species, individuals growing in the hills show different chromosome number from those growing in the plains. The hill varieties have 2n = 44, whereas the plain varieties have 2n = 46. Among the Hawaiian species, P. lilifolia shows two different chromosome numbers. Specimens collected from Mt. Kaala (Oahu) have n = 24, while those from Waikamoi (Maui) have n = 22. Although the ecological conditions in those two places seem to be similar, the number of Peperomia species found at Waikamoi is larger than that found at Mt. Kaala. Whether edaphic factors may play any role in this matter is not known. This type of behavior is not confined to the genus Peperomia, but is also found in genera such as Cardamine (Lockvist, 1947) and Caltha (Leoncini, 1951).

The differences in chromosome number between Skottsberg's (1955) report and the present paper can be expected, since Skottsberg's counts were based on the somatic number, while the present report has been based on the gametic number. According to Sharma and Sharma (1956) and Sharma and Bal (1956) variations in chromosome number within different cells of the same tissue within the same individual have been noted as a common occurrence in vegetatively propagated plants. In plants whose propagation is mainly through vegetative means, speciation may be brought about through changes in the somatic tissue

(Sharma, 1956). This process provides an additional method of speciation in plants whose reproduction includes both sexual and vegetative means. Such changes in somatic cells may involve alterations of chromosome number. Most of the Hawaiian species have the ability to reproduce vegetatively by means of stolons. Therefore, variation in somatic chromosome number as reported by Skottsberg (1955) may have occurred. Perhaps, this condition causes the existence of variations in Hawaiian Peperomia.

Meiotic irregularities occurred in the pollen mother cells of the eight species studied. These abnormalities were observed mostly during chromosome disjunction, except in P. cookiana where also severe contraction of chromosomes occurred in M I and M II. The most common irregularities observed were: 1. precocious disjunction and 2. lagging chromosomes and bridges with or without fragments. The present data do not provide any clue as to whether these abnormalities can be considered as characteristic for the Hawaiian species.

The high percentage of abnormalities in 18 species of Peperomia led Blot (1961) to conclude that their abnormalities are due to the process of a meiotic phase which progresses more or less rapidly. Further, he explained that the metaphase plate is not large enough to accommodate all chromosomes in a single plane. Somewhat similar conditions were found in the genus Pinus (Saylor and Smith, 1966). The long and large Pinus chromosomes suggest their

susceptibility to disturbances in chromosome movement, therefore causing the characteristic abnormalities throughout the genus. Similar abnormalities have been reported from other species with large chromosomes (Darlington, 1937). Applying such hypotheses to the Hawaiian species, it could be expected that all species observed would show these abnormalities, but only eight out of 20 species observed possessed them.

The occurrence of the bridge-fragment configuration raises the question whether this configuration is a result of inversion hybridity such as reported for maize (McClintock, 1931) and Paeonia (Stebbins, 1938), or of chromatid breakage and fusion such as reported for Trillium (Matsuda, 1950), Lilium, Paris (Haga, 1938, 1953), Paeonia, and Tradescantia (Lewis and John, 1966). Sharma and Bhattacharyya (1959) did not mention the occurrence of the bridge-fragment configuration among the abnormalities in the species they observed, while Blot (1960) did not specify the inclusion of the bridge-fragment configuration among the abnormalities caused by mechanical disturbances. A good diakenesis phase which enables one to observe chiasmata formation, which, in turn, can be used as evidence for one or the other explanations was not observed in the present investigation. Also, it is impossible to determine the constancy of the fragment size, since the percentage of occurrence is quite low. Thus, no attempt has been made to interpret this bridge-fragment configuration.

However, the high percentage of laggards, as well as the occurrence of precocious disjunction observed in meiosis, suggest the probability of hybridization in the species involved. A similar condition was observed in a putative hybrid in the genus Pluchea (Cooperider and Galang, 1966).

Therefore, on the basis of available data, it can be suggested that numerical alteration of chromosomes, polyploidy, and hybridization have played important roles in the evolution of the Hawaiian species of Peperomia. Taking Skottsberg's (1955) data into account, it is suggested that alteration of chromosome number of the somatic cells may also occur in Hawaiian Peperomia. Most likely the alteration of the chromosome number in the body cells can be carried on through vegetative propagation, since this type of propagation has been found in wild populations of the Hawaiian species.

CHAPTER 7.

LEAF ARRANGEMENT

Introduction:

In the family Piperaceae, Peperomia is the only genus in which alternate, opposite, and whorled arrangements of leaves are found. The rest of the genera in the family have alternate leaves only.

The majority of the Hawaiian species belong to the section Vercicillatae of the subgenus Sphaerocarpidium, and to the subgenus Hawaiiana, with an opposite or whorled arrangement of leaves. The five Hawaiian species which bear alternate leaves only are: P. alternifolia, P. degeneri, P. dextrolaeva, P. haupuensis, and P. oahuensis; these species belong to the section Alternifoliae of the subgenus Sphaerocarpidium. Miquel (1843) pointed out that on the same branch of some Peperomia, the leaf arrangement may be alternate, opposite, or whorled. This condition is also true in some Hawaiian species (Yuncker, 1933). Pertinent to the whorled arrangement of Peperomia leaves, Bravais and Bravais (in Schoute, 1925) recognized a normal Fibonacci spiral in P. blanda and P. inaequalifolia; these usually have ternary and quinary/octonary whorls, respectively. These authors felt that the whorled arrangement was better interpreted as an approximation of certain members of a genetic spiral to one another. This interpretation was supported by Miquel (1847).

Goebel (in Schoute, 1925), working with the same species, was inclined to consider the whorls of Peperomia as derived by contraction from a decussate phylotaxis. Controversy has since developed as to the nature of a whorl in this genus. Schoute (1925) supported Bravais and Bravais (in Schoute, 1925), and suggested the term "binding whorls" for the whorled arrangement in Peperomia. Rousseou (1927) concluded that although from the external morphology the occurrence of three to five leaves at each node represent a whorled arrangement, anatomically this is not so. Based on the nodal anatomy of 12 Peperomia species, Murty (1959) provided evidence to support both Bravais and Bravais's (in Schoute, 1925) and Goebel's interpretation. He indicated that in P. reflexa and P. fenzlei, the whorled condition may have been derived from opposite decussate leaves, whereas in P. cniapas, and P. prostrata, the opposite condition appears to be derived from the spiral arrangement. In studying the Hawaiian species, both de Candolle (1913) and Yuncker (1933) agreed with Miquel's interpretation (1843).

The occurrence of more than one phyllotactic system within a species is not uncommon. Deschatres (in Cutter, 1963) reported the occurrence of both bijugate and Fibonacci spiral phyllotaxis in Sedum elegans. More recent works have described the presence of more than one system of phyllotaxis in Dryopteris dilatata (Cutter, 1963) and Michelia fiscata (Tucker, 1962).

Results and Discussion:

All preceding studies were made of mature stems. In the present paper, the nature of leaf arrangement is investigated from the formation of leaf primordia in the shoot tip. Ten species, representing three subgenera, and two sections were investigated.

The vegetative shoot apices of Peperomia species are very small. If the inflorescences are borne terminally, the difference between the vegetative and the reproductive bud can only be determined under the dissecting microscope. In either the vegetative or reproductive bud the apex is enclosed by the involute young leaves (Plate 5A).

The vegetative shoot apices of mature plants of these 10 species were dissected and the arrangements of leaf primordia were plotted in diagrams. These diagrams were compared with the nodal vascularization. Serial transverse sections of the nodes were investigated to study the departure of leaf traces.

The leaf arrangements of the species studied are shown in Table 8.

The arrangement of leaf primordia in P. reflexa is shown in Fig. 1. The leaf primordia are arranged in opposite decussate pairs. Fig. 2 demonstrates the nodal vascularization of the mature stem. The vascular bundles of each pair of opposite leaves depart from the stem at the same level. The leaf traces for the pair of leaves arranged at right angles to the first pair, leave the stem at a slightly higher level than those of the first pair.

Thus, the apparently whorled arrangement in P. reflexa, as viewed at the shoot apex is seen to be actually an opposite decussate system. This condition is confirmed by the type of vascularization in the node.

Both P. oahuensis (Fig. 1b) and P. alternifolia (Fig. 1c) of the subgenus Sphaerocarpidium, section Alternifoliae, have the same arrangement of leaf primordia. In these two species, the leaf primordia are arranged in 1/2 phyllotaxis, although a slight deviation from this angle was also observed. This system is reflected in the alternate arrangement of the mature leaves.

The arrangement of leaf primordia in P. membrancea, P. leptostachya, and P. sandwicensis of the subgenus Sphaerocarpidium, section Verticillatae are shown in Fig. 1e, 1f, and 1g, respectively. Each pair of leaves is subopposite. They differ from P. reflexa in that one member of the pair is slightly smaller than the other. Basically, these three species have decussate-subopposite leaves; however, one member of each pair develops earlier than the other. From the study of the vascularization of mature plants, it is seen that each leaf trace in a node diverges from the stem at a slightly different level than the other three.

These three species have whorled arrangements of leaves in mature plants. It is interpreted then, that the whorled arrangement is a result of contraction of the decussate-subopposite leaf system shown at the shoot apex.

P. latifolia (Fig. 1d) has a leaf arrangement intermediate between a spiral system and a dorsiventral system. The members of each pair tend to be

subopposite to one another. However, the second pair is not quite at right angles to the first pair.

From the cross-section of the node with subopposite leaves, it can be seen that the departure of the two leaf traces does not occur at the same level. One member departs earlier than the other. One more often finds an alternate arrangement in mature plants than an opposite arrangement. The elongation of the internodes is quite irregular, especially in the upper part of the plant.

Spirally arranged leaf primordia are found in P. eekana (Fig. 1i) and P. lilifolia (Fig. 1h) of the subgenus Hawaiiana. The phyllotaxis in the shoot apex appears to be 2/5.

The vascular bundles in the node depart at different levels. Thus, the whorls of these species are the result of a contraction of a spiral system.

The leaf primordia in P. hypoleuca, of the subgenus Hawaiiana, are arranged dorsiventrally, resembling those of P. oahuensis and P. alternifolia, but the former species differs from those two in having opposite leaves in mature plants.

It is obvious, as shown by the nodal anatomy, that the two members of each pair of leaves have traces which depart from different levels. A different picture is seen in serial sections of the shoot tip in paraffin method. The leaf primordia of P. hypoleuca are not arranged dorsiventrally but spirally (Plate 5B). Apparently, in the older primordia this position is

shifted to a dorsiventral arrangement. Therefore, the opposite arrangement of mature leaves in these species is merely a contraction of an alternate system.

The above results show that in the species observed, really, only P. reflexa has opposite leaf primordia. The rest of the species have either subopposite or spiral arrangements of leaf primordia.

Pertinent to the whorled arrangement of Peperomia leaves, Miquel (1843) suggested that the whorls are the result of the atrophy of internode elongation. The failure of internode elongation in Halophila and Mondo causes an oscillating leaf arrangement, which Eckardt (1937) called the "halophila" type of phyllotaxis. In this type of phyllotaxis, the successive pairs of leaves lie in two planes; the members of each pair arise singly but the internode between them does not elongate resulting in a whorled arrangement. While the leaf arrangement in these plants is similar to that of the whorls in Peperomia, the latter has no sheathing leaf bases.

Anatomically, the failure of internode elongation can be demonstrated in longitudinal sections of stems. Two factors may possibly be responsible for this condition, i.e., cell elongation and cell division. The size of parenchyma cells in the nodal region of the mature stem (Plate 5C) is smaller than that of the parenchyma cells of the alternate internodes. This indicates that cell elongation in the internodal region has been suppressed, causing the closure

of two corresponding nodes. The average number of layers of these parenchyma cells would be equal to the number of layers of the internodal parenchyma cells if cell elongation was the only factor that operates here. However, these small parenchyma cell layers are fewer in number than the internodal parenchyma cells. This provides evidence suggesting that cell division in this region has been decreased. The decrease of both cell elongation and cell division results in the failure of internode to elongate. If only alternate internodes elongate, this condition will give rise to the subopposite condition, such as that in P. hypoleuca. Failure in elongation of two or more internodes caused the whorled arrangement of the leaves in P.membranacea, P. leptostachya, etc.

According to Schoute (1925), the whorls in Peperomia are the product of binding or attachment of the separate primordia to their lateral neighbors. The binding may probably appear at any stage of development. Further he says that the binding whorls are formed out of the spirally arranged vegetative leaves. Among the Hawaiian Peperomia observed, P. lilifolia and P. eekana show spirally arranged primordia. The number of leaves in each whorl varies. From the fusion of the bases of their petioles, it is observed that no more than three leaves are directly connected through lateral fusion. The rest of the members of the whorl are slightly higher. Apparently, then, the whorls in Peperomia are the product of at least two processes: first, the lateral fusion, probably due to lateral growth of the primordia resulting in lateral "binding" as described by Schoute (1925); second, the failure of internode elongation,

evidence for which is shown in the node of leaf insertion, and from the size and the number of layers of parenchyma cells at the nodes. Still there is the third possibility, i.e., the fusion of the leaf traces between the adjacent leaves, which is indicated by the fusion of the vascular bundles in a mature stem.

From the developmental standpoint, Priestley and Scott (1933) regard the spiral system as derived from the successive appearance of new primordia. On the other hand, in the decussate system, the two primordia appear simultaneously. According to them, a slight variation in time and place of origin of the individual primordia changes the system. This type of a change is found in P. hypoleuca, and P. latifolia, in which both types of primordial arrangements are found. The majority of shoot apices in P. hypoleuca have primordia which are arranged dorsiventrally; however, it is not uncommon to find primordia which tend to appear in succession at a divergence less than 1/2. On the contrary, the majority of the shoot apices in P. latifolia have a spiral arrangement, but dorsiventrally arranged primordia are also found in this species. A similar condition of shifting of primordial position might be expected in any other system found. As mentioned by Priestley and Scott (1933), the decussate system in dicotyledons is a stable system. The constant number of leaves in a whorl of P. reflexa support such a hypothesis.

In discussing whorls of more than two numbers (n), Priestley and Scott (1933) concluded that 2n leaf primordia should be growing at the apex

simultaneously. They give evidence that the larger the number of leaves in a whorl, the greater the size of the apex. On the other hand, Hageman (1960 showed that the larger the youngest leaf at the time another leaf is initiated, the fewer the leaves present in terminal buds. Thus, the size of primordia determines the number of primordia present on the terminal bud. Studies of serial cross sections of shoot apices of P. hypoleuca (Plate 5B) support the views of Hageman (1960).

Among the Hawaiian Peperomia observed, two species are alternate, two have the tendency of being opposite, and the rest have the tendency of being whorled. Cutter (1963) suggested that temporary or permanent changes in phyllotaxis may be governed by progressive changes in the rates of growth. From the present studies, it appears that the whorls in Peperomia, although they can be explained developmentally, have the tendency to be genetically fixed. This conclusion is drawn from the fact that both species with alternate leaves and whorled leaves grow under the same environmental conditions. In all species with whorled arrangements, it is noted that the whorls are always associated with the maturity of the plants. Thus, the young stem of the mature plant has whorled leaves while that of the seedling does not.

The effect of gibberellic acid on stem elongation of various kinds of higher plants has been shown (Meyer et al, 1960; Sachs et al, 1960). Cutter (1963) suggests that GA treatment resulting in internodal elongation can affect the angle

of divergence in Dipsacus, thus changing its phyllotaxis. Therefore, treatment with GA may offer some evidence bearing on a possible explanation concerning the phyllotaxes in the genus Peperomia. With the investigation of larger samples it is also possible that other phyllotaxes which are not reported at present might be found.

CHAPTER 8.

LEAF ANATOMY

Introduction:

The leaves of all Hawaiian Peperomia species are simple, palmately veined, with entire margins. No species with peltate leaves were found. Although the shape and size of leaves varies within many of the species, these characters may be used for taxonomic purposes (Yuncker, 1933). In most Hawaiian species, a red pigmentation of the lamina and the stems has been observed (Yuncker, 1933). Yuncker (1933) suggested that the pigmentation is of no value in taxonomic classification of the genus. His assumption was based on the fact that the occurrence of such pigment varied in different plants of the same species as well as in different parts of the same plant.

Skottsberg (1947) has commented upon the rather monotonous anatomical structure of the genus Peperomia; however, some variations do occur. A few studies have been made on the anatomy of this genus. Jaderholm (1898) made the first comparative anatomical study of leaves of South American species of Peperomia. Yuncker and Gray (1934) made similar studies of twelve Hawaiian species, while Murty (1960) studied twelve species collected from all over the world.

There are variations in the number of vascular bundles entering the leaves in the genus Peperomia. From a study of the nodal anatomy, Weiss (in de Bary,

1884) inferred that there are one (P. galiodes), three (P. brachyphylla), seven (P. incana) or 13 (P. variegata) leaf traces. Unaware of Weiss's (1876) work, Sinnott (1914) described the three trace leaves of Peperomia as representing the primitive condition in the family Piperaceae. Rousseau (1927) described the traces in P. verticillatae and P. incana as being three, and seven to nine, respectively. Three-trace vascularization was also found in P. pellucida, P. fenzlei, P. reflexa, P. comarapana, P. blanda, P. cniapas, and P. prostrata, whereas some unidentified Peperomia had five traces (Murty, 1960). He also noticed that the maximum number of leaf traces in plants with opposite or whorled leaf arrangements is three.

Results and Discussion:

The present study includes 12 species whose leaf characters have not been studied previously. The outline of the lamina in the Hawaiian species varies from orbicular (P. cookiana, P. latifolia, P. remyi, and P. waikamoiana), to ovate-lanceolate (P. alternifolia, P. hypoleuca, P. kokeana, P. koolauana, P. leptostachya, P. lilifolia, P. macraeana, P. membranacea, P. oahuensis, P. reflexa, P. remyi, P. sandwicensis, P. trichostigma), to obovate (P. erythroclada, P. expallescens, P. hawaiiana, P. hesperomannii). A graphic presentation of leaf size and shape of some species is given in Fig. 3. The graph was obtained by plotting the average lengths and widths of ten mature leaves from at least three different collections made in the Hawaiian Islands. It appears that several

of the species can be clearly differentiated in terms of leaf dimensions. There are three groups of plants segregated according to leaf dimensions, i.e., the large ovate-leaved P. hirtipetiola group, the intermediate-leaved P. hypoleuca group, and the small leaved P. reflexa group. Considerable variations in size and shape of leaves occur in P. cookiana, P. leptostachya, and P. latifolia. These variations in leaf shape are shown in Fig. 4.

According to Hutchison (1966) leaf dimorphism is common in many tropical new world Peperomia species, which is probably related to seasonal rains. He proposed a hypothesis that the smaller leaves were produced in dry seasons, whereas much larger leaves were associated with rainy seasons. He believed the phenomenon is widespread in the genus. Therefore, according to him, when the living plants are more carefully studied many so-called new species will fall into synonymy.

It has been mentioned that among the Hawaiian species, considerable variation in size and shape of leaves occurs in P. cookiana, P. leptostachya, and P. latifolia. There are two possible causes which induce this variation. First, the difference in the microenvironmental condition and second, the genetic changes in the somatic tissue as outlined on p. 42. The microenvironmental condition seems to play an important role in changing the leaf shape of these species, because this kind of variation is shown even in plants growing in the same area, but in different rock crevices. Therefore, the leaf variation

may occur at any time of the year depending entirely on the variation of the microenvironmental condition. The tremendous variation in size of plant and leaf in populations of P. leptostachya due to a high production of stolons may have been caused by chromosomal changes in the somatic tissue. Further study of somatic chromosomes is needed to substantiate such an assumption.

Many of the Hawaiian species have trichomes on the abaxial surface of the blade, sometimes accompanied by more extensive development of those along the adaxial surface of the major veins (P. eekana, P. ellipticibacca, P. expallescens, P. erythroclada, P. helleri, P. hirtipetiola, P. hypoleuca, P. latifolia, P. lilifolia, P. macraeana, P. remyi, and P. sandwicensis). Only P. alternifolia, P. dextrolaeva, P. globulanthera, P. hawaiiensis, P. hesperomannii, P. membranacea, and P. oahuensis appear glabrous; however, in all these species trichomes were found at the tips of the leaves (Plate 5D).

The trichomes are uniseriate-multicellular, and vary in length and shape. According to Yuncker (1933) the number, location, size, and position of these trichomes provide diagnostic characteristics. In his key and description of Hawaiian species he used the term "hirsute" for the trichomes from 0.5 - 1.5 mm in length, and the term "hirtellous" for those which were smaller. In the present studies, leaves of 26 species representing all the subgenera and sections occurring in the Hawaiian Islands, were investigated. The leaves of these species were cleared and the trichomes of representative leaves

were drawn by projection methods. Both the midrib and the area between the midrib and the second major vein are shown for each species (Fig. 8, 9, 10, 11, 12 and 13). From the study of the drawings in Fig. A and B, it is clearly apparent that there are at least four types of trichomes found in the Hawaiian species. The long (1.70 mm) trichomes with undulate outlines are shown in Fig. 5A (P. hirtipetiola), the moderately (1.00 - 1.50 mm) sized ones shown in Fig. 5B (P. lilifolia, P. ellipticibacca, P. expallescens), the shorter (0.50 - 1.00 mm) shown in Fig. 5A and 5B (P. eekana, P. hypoleuca, P. waikamoiana), and the shortest (0.50 mm) ones shown in Fig. 5A and 5B (P. latifolia, P. macraeana, P. kokeana, P. oahuensis, P. remyi). P. leptostachya, P. cookiana, and P. sandwicensis have mixtures of the third and fourth types, above. This mixture was observed in one leaf (P. leptostachya), or found in different varieties of the species (P. cookiana, P. sandwicensis). Plants of P. latifolia which were collected from higher elevation showed longer trichomes (Fig. 5Bj) than plants collected from lower elevations. P. reflexa differs from the rest in having very short (0.2 mm) trichomes consisting of two cells at the most; the walls of these are very thick (Fig. 5B). On the surface of each trichome of P. hirtipetiola, P. macraeana, P. lilifolia, and P. remyi, there are cuticular warts (Plate 5E).

Also in the apical cells of these trichomes, the thin membrane of the primary wall was observed (Plate 5F). P. cookiana is very characteristic in

having swellings of the walls at the region in which two cells of the trichome are joined. Branched trichomes such as those reported by Skottsberg (1956) in P. mammularoides were not observed in the Hawaiian species. Table 9 gives the size and the average number of cells of the trichomes in each species.

Yuncker (1933) noted the occurrence of stomata on the adaxial surface of P. leptostachya and Pant and Banerji (1964) reported the same in P. reflexa. In the present investigation stomata were found only on the abaxial surface except in P. leptostachya, in which they are also present on the adaxial surface. In this species there were 14 stomata per 100 epidermal cells on the abaxial surface and only 2 stomata per 100 epidermal cells on the adaxial surface. These stomata are scattered and irregularly oriented among the epidermal cells. According to Metcalfe and Chalk (1950), the stomata of Piperaceae are either surrounded with a rosette of numerous epidermal cells or are of the cruciferous or anisocytic type, i.e., a stoma with three unequal subsidiary cells. The occurrence of anomocytic stomata, i.e., a stoma with no subsidiary cells, in P. pellucida, and tetracytic stomata, i.e., a stoma with four subsidiary cells, in P. reflexa was reported by Pant and Banerji (1964). The present study confirms the previous reports. Stomata with rosettes of epidermal cells were found in P. lilifolia, P. ellipticibacca, P. eekana, P. hesperomannii, P. helleri, P. hirtipetiola, and P.kahiliana (Fig. 6e, f). The rest of the species have both anomocytic and tetracytic types (Fig. 6a, b, c, d). The

guard cells are usually raised slightly above the surrounding epidermal cells. The thin, sharp, protrusion of cutin occurring on the guard cells can be clearly seen in transverse sections (Plate 6A). The circular outline of the guard cells in P. reflexa can be used to distinguish this species from the rest of the Hawaiian species. Table 10 gives the number of guard cells per 100 epidermal cells and the size of the guard cells in individual species.

All of the Hawaiian Peperomia species have multiseriate epidermal cells (Haberlandt, 1914; Foster, 1949). The outermost layer of epidermal cells may have either a thick or a thin cuticular layer. The size and shape of these cells might well be used for diagnostic purposes in recognizing species. P. hesperomannii, P. helleri, P. kalihiana, and P. lilifolia have relatively small (±50 μ in diameter) outermost epidermal cells, whereas the rest of the species have large (±100 μ in diameter) ones. In many cases there is a striation of the cuticular layer. The lateral walls in surface view may be straight in outline as in P. oahuensis, or undulate as in P. hirtipetiola.

Three-celled "glandular hairs" (Jaderholm, 1898) or "hydathodes" (Johnson, 1914) which resemble those of Piper betel (Chibber, 1912) were found scattered on both adaxial and abaxial surfaces of all species observed. The term "glandular hairs" is preferred to "hydathodes" in this paper, because evidence of secretion of water from these structures cannot be given at this moment. Skottsberg (1947) reported that the glandular hairs on the adaxial surface of the leaves of P. barteroana and P. tristanensis consist of two cells only, while those of the abaxial surfaces have three cells.

The three-celled glandular hairs were found in all Hawaiian species on both surfaces of the leaf. The basal cell is large, thin-walled, with a relatively small nucleus. The short stalk cell has dense cytoplasm and a moderately sized nucleus. The head or scale is globular or hemisperical with a large nucleus and thin wall. A very thin cuticle covers the head. According to Haberlandt (1914) the head is responsible for the secretion of water, the stalk is the mechanical component, and the base connects the rest of the glandular hair with the adjoining epidermal and the hypodermal cells. In some species a single long cell connects the basal cell of the glandular hair with palisade cells. The shape and the size of the head varies in several species. Head cells of discoid shape were found in P. lilifolia, and head cells of hemisperical shape in P. erythroclada.

One or two epidermal layers, which are located just beneath the outermost layer, consist of small cells which are flattened radially. The rest of the epidermal cells are cylindrical and oriented perpendicular to the surface of the leaf. These multi-layered cells are considered to be water-storing cells. In general, the cells are characterized by their thin walls and the absence of pits (Haberlandt, 1914). Habelandt's finding was confirmed by Skottsberg (1947). However, in the present study, the presence of pits in the walls of the epidermal cells was detected (Plate 6B). These are especially striking in the thick walls of the collenchymatous epidermal cells of some species. Metcalfe and Chalk (1950) referred to the water-storing cells as a hypodermis. This term will be used here.

In P. berteroana many of the hypodermal cells are strengthened by delicate "cross bands" (Skottsberg, 1947). He reported that these thickenings are developed in the short cells beneath the outermost layer and rarely in the distal part of the lamina. He compared those "cross bands" with the spiral bands in the water-storage tracheids in the velamen of orchids (also a multiple epidermis) reported by Haberlandt (1914). Further, he stated that these "cross bands" are not identical with those in the radial wall of the hypodermal cells which according to Haberlandt (1914) are caused by the withdrawal of water from these cells. Jaderholm (1894) and Yuncker and Gray (1934) did not mention the occurrence of such "cross bands". The present investigation supports Habelandt's (1914) latter explanation. The presence of the "cross bands" is readily shown in P. leptostachya and P. reflexa, in which up to six layers of hypodermal cells were observed (Plate 6C). Both species often grow in more arid conditions than the rest of the Hawaiian species. They are easily noticeable in the samples of the two species taken from dry areas. These "cross bands" are absent in the fresh section of the same species grown in moist habitats. However, by letting the same plants lie for a few days at room temperature, the "cross bands" in the hypodermal cells developed.

Oil cells (Haberlandt, 1914; Becker, 1931), round in shape, containing a yellowish brown substance are scattered in the upper layer of the hypodermal cells, as well as in the region of mesophyll. In contrast to the Hawaiian species, Murty (1960) reported that in most species observed by him, these

cells were confined to the abaxial surface of the lamina. Further, he stated that these oil cells contained prominent nuclei and cytoplasm. Of these Hawaiian species studied in this thesis, this condition was found only in young oil cells (Plate 6D). In such a cell the nucleus was large and the cytoplasm was homogeneous. As the cell increased in size, oil droplets developed which later fused with each other (Plate 6E), and finally formed a single large oil compartment which occupied the whole space of the cell (Plate 6F). In this stage the nucleus started to be disorganized and the cytoplasm was found along the cell wall. Plate 9C demonstrates the thickness of the oil cell wall in which the nucleus as well as the cytoplasm could no longer be detected. Yuncker and Gray (1934) described these oil cells as "mucilage glands" and found them in only three of the 12 Hawaiian species they observed. They did not indicate the location of these cells in the leaf. The absence of oily substances in these cells, probably related to action of the fixative used, may have caused Yuncker and Gray to be unable to detect them in several species that they observed. According to Haberlandt (1914) the contents of such cells in many Zingiberaceae and Piperaceae is exclusively of excretion in the form of ethereal oil. According to Thorne (1966) the order Annonales is characterized by having ethereal oil cells; therefore, the family Piperaceae is included in it. In the present studies, the presence of oily substances was detected by staining with Sudan IV. Through sectioning of fresh stems, some of the oil cells were cut, releasing the oily substance out of the cells. As a

positive result a red coloration of this substance was detected. The uncut oil cells remained unstained; perhaps, the stain was not able to penetrate the cell walls, thus the oily substance did not come into contact with Sudan IV.

In many families, Berthold (in Haberlandt, 1914) observed that the excreted oil drops may be enclosed in protuberances of the suberin lamella. This statement was confirmed by Haberlandt (1914) who observed the same structures in Laurus and Asarum. The development of the oil cell has been followed in some species of Peperomia, Laurus, and Brachyphilla (Becker, 1931). According to him the formation of oil occurs exclusively in the protoplasm. Further, he stated that the basin of the oil storing cells does not consist of suberin, but is a protrusion of the cellulose lamella. A cellulose test, using aniline blue was made during the present study. A "stalk" which gave a positive cellulose stain with anilin blue, attaches this basin to the walls of the cells (Plate 7. 1A). In prepared slides, these cells are quite often empty.

Transverse sections of leaves of the Hawaiian species show differentiation of the mesophyll into palisade and spongy parenchyma. The palisade parenchyma consists of a single layer of small, almost isodiametric to slightly elongated, upright cells, which are more or less closely packed. These cells are rich in chloroplasts. Each cell of this layer contains a single druse crystal, the occurrence of which has not been previously reported for the Hawaiian species. In general, the single crystal is located in the

upper part of this cell, surrounded by the chloroplasts which are aligned against the cell walls, except for the upper wall. Schurhoff (in Haberlandt, 1914) suggested that the druses concentrate the light rays and then distribute them equally to all chloroplasts. Skottsberg (1947), however, does not agree with the view that the crystals in Peperomia species condensed the light, rather he believed that the druses refract the light in all directions. Whether this crystal has anything to do with light distribution, it is always associated with a palisade cells. The funnel shape of these palisade cells (Plate 7.1B) may give a better illumination for the chloroplasts located at the bottom of the cells. According to Noll (in Haberlandt, 1914) the funnel-like shape of palisade cells in certain species of Selaginella causes the cells to act as condensing lenses. The concave surface of this cell refracts the light that falls perpendicular to the leaf, giving an increased reflection toward the base of the cell; thus the chloroplasts which are located here are more brightly illuminated.

Under this layer, there is a single layer of rounded cells which connect the palisade layer with the spongy mesophyll (Plate 7.1B). These cells contain chloroplasts, which although fewer in number than those in the palisade cells, are more abundant than in the spongy mesophyll cells. Also starch grains are found in large numbers in these cells. Yuncker and Gray (1934) did not distinguish between the two layers and used the term "dense chlorenchyma" for both of them and considered them to constitute modified palisade tissue.

According to Jaderholm (1898) the rounded cells function in storage. He regarded this layer as a specialized layer of the spongy parenchyma which was not an assimilatory tissue. However, Skottsberg (1947) believed that this layer is active in assimilation. From his illustration of P. trichocarpa there is evidence that Haberlandt (1914) combined the two layers of cells as a photosynthetic tissue, although he does not discuss the matter. In discussing the photosynthetic system of Ficus and Aspidium he applied the term "collecting cells" for those connecting the palisade and spongy layers. He suggested that these cells function as receivers for the synthetic products from the palisade layer and also as transmitters for these products from the palisade layer to the rest of the system of translocation. The position of the rounded cells in Peperomia, as well as the number of starch grains present in these cells, suggest that they are "collecting cells" similar to those of Pinus and Aspidium.

The spongy parenchyma is well developed and occupies 1/3 to 4/5 of the thickness of the leaf. It consists of four to six layers of rounded (P. leptostachya type), or armed (P. membranacea type) cells, with small or large intercellular spaces. The species having the rounded spongy cells were collected from the arid habitats, while armed spongy cells are found in the species grown in moist habitats. The size of these cells is four to five times bigger than the palisade and the rounded collecting cells. Cell wall thickness varies among the species. The primary pit fields are well developed in the

region where the cells come into contact. These cells contain few chloroplasts and starch grains. Skottsberg (1947) reported the occurrence of crystals in this layer; however, none were found in the species examined by me. In P. cookiana, P. hesperomannii, P. hypoleuca, P. latifolia, and P. sandwicensis, many cells of this layer contain anthocyanin which give a red coloration to the lower side of the leaf. This phenomenon is common in many shade plants (Haberlandt, 1914). The importance of anthocyanins in the metabolism of vegetative plants organs has been suggested to be: in the protection of chlorophyll from strong and harmful insolation (Bunning, 1948), in influencing the thermal economy of plant tissue (Lippmaa, 1924), in the reduction processes of carbon dioxide (Noack, 1922), in plant respiration (Talladin, in Blank, 1958; Stilles, 1936), and as a hydrogen acceptor (Reichel and Burkart, 1938). Others have opposed these preceding hypothesis, such as Engelman (in Blank, 1958), Seybold (1942), and Molisch (1926). Frey-Wyssling (1942) regarded the anthocyanins as waste products which are excreted in cell sap. Further substantiation concerning the formation of these pigments in plants is needed in order to determine their roles in the plant and their ecological significance (Blank, 1958). Light, temperature, drought, nutritional deficiency are among factors affecting formation of anthocyanins in plants. Although some plants are able to form anthocyanins in the dark (Bunning, 1948; Frey-Wyssling, 1942; Blank, 1958), the majority of plants form anthocyanins in their organs only under strong illumination (Gimesi et al, 1952; Kosaka, 1932). It is noteworthy

to observe the development of this coloration in P. hypoleuca, P. hesperomannii, and P. sandwicensis. These species produce two types of plants; one has entirely green leaves, while the other has a red coloration. The green leaved plants and the red leaved plants grow side by side; most likely they have the same macroenvironmental conditions. The sizes of these plants are approximately the same and both have inflorescences of the same degree of maturity. These three species usually grow under trees in shady places. P. hypoleuca and P. hesperomannii are found in cold and wet areas, whereas P. sandwicensis is usually found in warmer, more arid regions. Whether the difference in coloration is a temporary one or a fixed genetic phenomenon which is governed by a single gene, can only be determined by following the growth cycles of these plants under controlled greenhouse conditions. The development of anthocyanins in P. reflexa, P. leptostachya, and P. latifolia was also observed, but they are never as abundant, and the plants are never as intensely colored as in the former species. More likely the development of these substances is governed by drought or nutrient deficiency because only leaves located in the lower part of the plant show this coloration.

The midvein in P. hesperomannii, P. hirtipetiola, P. hypoleuca, P. lilifolia, and P. remyi is raised on the abaxial surface of the leaf and there is a groove similar to that found in the petiole on the adaxial surface of the blade. The hypodermis is limited to one layer in this region. In the

preceding species the vascular bundle of the midvein is well developed, surrounded by a layer of small parenchyma cells. Plates of larger, rounded parenchyma cells extend from the bundle sheath toward the abaxial epidermis (Plate 7.1C). One to three layers of small collenchyma cells are located between these parenchyma cells and the epidermis. In P. latifolia, P. leptostachya, P. reflexa, P. oahuensis, P. alternifolia, and P. sandwicensis no adaxial groove was found. The vascular bundle of the midvein is poorly developed and the collenchyma layer is absent. In these species the palisade layer forms a ridge above the midvein (Plate 7.1D).

As viewed in cross section the margin of the leaf is either tapered (P. alternifolia type), blunt (P. leptostachya type), or revolute (P. hirtipetiola type). In general, one layer of parenchyma cells is located between the upper and the lower epidermis in this region.

For petiole studies, sections from both the proximal and of the petiole immediately above the point of insertion of the leaf, and from the distal end immediately below the lamina have been investigated, and a few samples are illustrated (Plate 7.2A, B, C, and D).

The petioles are somewhat flattened dorsiventrally, as seen in cross-sections (Plate 7.2A). In most species, there is a groove on the adaxial side, and in some species wings develop from the lateral surfaces. The epidermal cells are covered by cuticle; trichomes and glandular hairs are present. The cells of the cortex are collenchymatous toward the center. Quite often,

a layer of parenchymatous cells containing anthocyanins lies beneath the collenchyma layer. Oil cells and crystals of calcium oxalate are numerous in the cortex. Besides the solitary rhombohedral and octahedral (prismatic, pyramidal) crystals, there are compound structures, the druses and combinations of prismatic and pyramidal crystals present (Fig. 7).

There are usually three vascular bundles in the petiole, with the median bundle the largest. Petioles with one vascular bundle were observed in some leaves of P. waikamoiana, and some with five vascular bundles in P. dextrolaeva. Anastomosis and dichotomy of the vascular bundles in the petiole have not been observed. Upon entering the leaf blade, the marginal bundles divide, forming the palmate venation of the blade. The central veins are approximately parallel with the small lateral veins spreading toward the margins with numerous irregular anastomoses. Approaching the tip of the leaf, the major veins branch with the size of the tracheary element file becoming smaller. On the other hand, the two most marginal veins consist of only one file of tracheary elements. Toward the tip of the leaf, these bundles consist of four or five files of tracheary elements, resulting from the consolidation of the veinlets. At the tip of the leaf, the median bundles and the two marginal bundles fuse. The perforation plates of the tracheary elements in the bundles are simple and obliquely oriented.

CHAPTER 9.

STEM ANATOMY

Introduction:

From an anatomical point of view, the family Piperaceae, to which Peperomia belongs, is placed among the anomalous dicotyledons (de Bary, 1884; Balfour, 1958; Solereder, 1908). In describing the structure of the axis in the family Piperaceae, Solereder (1908) distinguished four types of vascular bundle arrangement. The genus Peperomia can be distinguished from the rest of the types in having vascular bundles scattered in the ground tissue. Ironside (1911) and Hoffstadt (1918) suggested affinities of the family with monocotyledons, on the basis of scattered vascular bundles in the internodes. After studying the vascular anatomy of 11 species of Peperomia and eight of Piper, Weiss (in de Bary, 1884) concluded that in the Piperaceae there is a transition from the monocotyledonous to the dicotyledonous arrangement of bundles. Of course, it must be recognized that there is no tenable classification of plants as dicotyledons and monocotyledons on the vascular bundle arrangement alone as there are many exceptions in both taxa (Esau, 1960).

Other features generally found in monocotyledons which are also present in Piperaceae are the presence of a single cotyledon in some Peperomia seedlings (Hill, 1906), and a single prophyll (Balfour, 1958).

In some Peperomia, the vascular bundles are arranged in two or more concentric rings, and lack a vascular cambium; thus no secondary growth has been observed (de Bary, 1884; Johnson, 1914; Skottsberg, 1947; Yuncker, 1933). According to Weiss (in de Bary, 1884), all bundles in Peperomia are leaf traces, although Moldenhauer (in de Bary, 1884) concluded that the bundles are partly cauline; this latter conclusion is supported by Yuncker (1933).

The general anatomy of some Peperomia species has been discussed in several papers (Johnson, 1914; Solereder, 1908; Yuncker, 1933). By far the most detailed account is that of Skottsberg (1947). He made an anatomical comparison between P. berteroana and P. tristanensis, and came to the conclusion that although the anatomy of the species are rather similar, some variations are present; he was able to use these differences to distinguish between the two species.

Results and Discussion:

Of the 47 described Hawaiian species, 12 species have been studied anatomically (Yuncker and Gray, 1934). The present studies include 12 species whose stem characters had not previously been recorded.

Utilizing Sachs' (1875) classification based on topographical continuity of tissues, the tissue system of Peperomia stem consist of epidermis (the dermal), collenchyma and parenchyma (the fundamental), and the primary phloem and xylem (the vascular system).

As viewed in cross section the internodes in P. alternifolia, P. dextrolaeva, P. hawaiiensis, P. hesperomannii, P. hypoleuca var. pluvigaudens, P. membranacea, and P. oahuensis have three or four wing-like ridges which are especially conspicuous in young stems.

A thin layer of cuticle covers the outer walls of the epidermal cells. In P. reflexa and P. leptostachya, the cutinization is heavy. These two species grow in arid habitats. In P. reflexa, Yuncker and Gray (1934) reported that the radia wall of the epidermis is also cutinized. The structure of the epidermal cells in P. reflexa is quite unique (Plate 8A), since the epidermal cells are circular andheavily cutinized radially. In P. sandwicensis, P. lilifolia, and P. latifolia the epidermal cells are almost isodiametric, whereas in P. hesperomannii, P. leptostachya, and P. cookiana, the length of the cells which are oriented vertically is about twice their diameter.

The epidermal cells are arranged in a single layer, except in P. hesperomannii, P. leptostachya, P. lilifolia, and P. sandwicensis where two layers of epidermal cells were observed. In those species, the inner layer consist of cells three to four times larger than those in the outer layer.

P. latifolia, P. lilifolia, P. sandwicensis, and P. reflexa have the smallest epidermal cells; their sizes average about 30 x 30 x 50 μ. P. expallescens, P. hesperomannii, and P. hirtipetiola have the largest epidermal cells measuring 70 x 70 x 90 μ.

Trichomes similar to those on the leaves were present in all species observed except in P. alternifolia, P. dextrolaeva, P. globulanthera, P. hawaiiensis, P. hypoleuca var. pluvigaudens, P. koolauana, and P. membrancea. The hairs do not persis in the older stem; thus, older stems of all species appear glabrous.

Three-celled glandular hairs resembling those on leaves were found in the epidermal layer of all species observed. The hairs are located in shallow pits. Like the uniseriate hairs, these glandular hairs are also absent in the older stem, leaving the pits as depressions in the epidermal layer (Plate 8B). As mentioned by Yuncker and Gray (1934), the glandular hairs on stems are less abundant than on leaves. In P. hypoleuca and P. hesperomannii, some of the epidermal cells contain small crystals of calcium oxalate.

A few layers of collenchymatous cells are well developed beneath the epidermal layer. The wall thickenings are of the angular type, i.e., the thickenings are localized in the corners. Variations in the numbers of layers were observed. In P. expallescens, P. cookiana, P. latifolia, P. leptostachya, P.alternifolia, P. dextrolaeva, P. membranacea, P. oahuensis, P. koolauana, and P. reflexa there are three to six layers, whereas P. eekana, P. ellipticibacca, P. hirtipetiola, P. hypoleuca, P. lilifolia, P. macraeana, P. globulanthera, P. hawaiiensis, P. hesperomannii, P. kalihiana, and P. kokeana have more than seven layers of collenchymatous cells. Johnson (1914) reported the absence of collenchymatous layers in P.hispidula. One layer of

collenchyma was observed in P. pellucida (Murty, 1960). Yuncker and Gray (1934) observed as many as 23 layers of collenchyma in P. rockii. The size of collenchyma cells gradually increased toward the center of the stem. Simple pit areas were observed in all species studied (Plate 8C).

The collenchymatous layer is followed internally by the parenchymatous ground tissue. The cells composing this tissue vary in shape, size, and wall thickness. Most of the species have small parenchyma cells averaging from 80 μ to 120 μ in diameter and 120 μ in length. P. hirtipetiola, P. hypoleuca, P. hawaiiensis, P. hesperomannii, P. latifolia, and P. membranacea have larger cells measuring 125 μ to 160 μ in diameter and 180 μ in length. The parenchyma cells in P. latifolia, P. ellipticibacca, P. sandwicensis, and P. expallescens have very thick walls (4 μ), while P. lilifolia, P. hirtipetiola, and P. membranacea have thin walls (1 μ). The rest of the species have medium thick walls (2 μ). The pit areas on the longitudinal walls are well developed (Plate 8D). Many (about 30 per face) pit areas are found in P. hesperomannii, P. sandwicensis, P. lilifolia, and P. hirtipetiola; P. latifolia, P. ellipticibacca, and P. expallescens have the smallest number of pit areas per cell (about 10 per face), and the rest of the species have intermediate numbers. Sometimes a group of pores was observed in each pit area, as represented by P. sandwicensis. The occurrence of such pit areas has not been reported by previous workers. According to Yuncker and Gray (1934) prominent intercellular spaces were only observed in P. leptostachya, and P. reflexa, whereas in other species the intercellular spaces were lacking or very small. In the

present study, all species exhibited intercellular spaces. P. latifolia and P. hesperomannii are among the species which have large intercellular spaces (Plate 8F).

In most cases the presence of anthocyanins was observed in the ground parenchyma cells. Quite often, the whole outermost layer of the ground parenchyma cells contained anthocyanins. A few chloroplasts were present in most cells. Starch grains were abundant, especially in those cells surrounding the vascular bundles. Various forms and shapes of crystals, either solitary or compound, were observed in the cells. Table 11 shows the occurrence of various crystals in each species observed. Crystals were not observed in P. reflexa and P. hawaiiensis. Comparison with fresh material was made in P. reflexa, but no crystals could be found there either. This observation confirms Yuncker and Gray's (1934) report. Comparison with fresh specimens in P. hawaiiensis is needed in order to determine whether or not crystals were present there but disappeared during the preparation of permanent slides.

Oil cells are found scattered among the collenchyma cells and ground parenchyma cells (Metcalfe and Chalk, 1960). Engler (1964) reported the occurrence of resin or oil canals in Piper but not in Peperomia. Solereder (1908) distinguished mucilage canals which are found only in the axis of the genus Piper from the secretory cells which are found in both leaf and axis of all the genera of Piperaceae. According to Yuncker and Gray (1934), among

the Hawaiian species studied by them, only P.oahuensis contained cells which were similar to Solereder's mucilage canals. Skottsberg (1947) reported the occurrence of oil cells in the stem of P. berteroana and assumed that these cells were of the same nature as the cells observed by Yuncker and Gray (1934). Skottsberg believed that these cells were similar to Solereder's (1908) secretory cells. The present investigation supports the view of Skottsberg. Oil cells were observed in the stems of all species included in the present study. The number of oil cells in the stem varies in different species. Oil cells were most abundant in P. hesperomannii, and least abundant in P. alternifolia. In P. hesperomannii, P. latifolia, and P.hypoleuca, these cells show a greater tendency to be associated with vascular bundles (Plate 9A). Approaching the nodes the number of the oil cells increases. In cross sections the shape and the size of oil cells are similar to those of the surrounding parenchymatous cells (Plate 9B). They can be distinguished easily in fresh sections of the stems by the color of the substance inside, but not so in prepared slides. However, in longitudinal sections, these cells differ from the surrounding parenchymatous cells in their size and shape. In general, the oil cells are shorter and more rounded in shape than the parenchyma cells.

Some explanations of the anomalous stem anatomy of the Piperaceae have been suggested (de Bary, 1884). Karsten (in de Bary, 1884) believed that all bundles are leaf traces. In comparing the course of the bundles in the woody and herbaceous Piperaceae, Weiss (in Bond, 1931) showed that the vascular

bundles of the genera Piper and Arthante are partly foliar and partly cauline. Hofstadt (1914), however, described the bundles of Piper mythesticum as of foliar origin. Another genus reported to have both cauline and foliar traces is Micropiper (Balfour, 1957). On the contrary, in the genus Peperomia, only bundles of foliar traces were found in the internode (Weiss, in Bond, 1931). Yuncker and Gray (1934) described the arrangement of the vascular bundles in the Hawaiian species they studied. In all these species, there were two rings of vascular bundles. The peripheral rings were considered to be strictly foliar, whereas the bundles of the inner ring were thought to be either basic central bundles or foliar traces. The present investigation, using P. hypoleuca as representative for the Hawaiian species, supports the view of Weiss (in de Bary, 1884). There is no procambium tissue developed below the apical meristem which will give rise to medullary bundles as shown in Micropiper (Balfour, 1957). One of the vascular bundles becomes inserted at the medullary region. At lower levels the number and the distribution of the vascular bundles vary. These bundles are sometimes irregularly distributed. In other cases, they are arranged more or less in two rings (Plate 9E). The joining of the members of the two rings may occur either at nodes or somewhere in an internode resulting in variation in the number of the bundles in the internode. Two bundles of the same ring may join (Plate 9F), or two bundles from different rings may fuse as shown in Plate 10A. There are also divisions of the bundle into two (Plate 10B), one of the branches then

joining another bundle (Plate 10B). Therefore, at the node, complex fusions and divisions of the bundles were observed. The union of the bundles of two or more leaves is interpreted here as one of the causes of the whorled condition in some Peperomia species. In comparing the nodal anatomy of Piperaceae, Rousseau (1927) concluded that the genus Piper formed the fundamental type of the family from which two types shown by other genera were derived. The genus Peperomia shows a major modification from the Piper type. On the contrary, Sinnott (1914) considered the genus Peperomia as representing the primitive condition for the family since Peperomia has trilacunar nodes while in Piper five to seven traces were observed. He believed that the trilacunar condition was the most primitive type in dicotyledons, from which other types of nodal anatomy have been derived. Based on his investigation in P. rubella, Murty (1960) supported Sinnott's (1914) view that by the disappearance of the lateral bundles, a three trace condition gives rise to one trace condition. Among the Hawaiian species, P. waikamoiana has one and three traces, and P. dextrolaeva has three and five traces. Fitting these conditions to currently accepted schemes of evolutionary patterns of nodal anatomy in dicotyledons (Carlquist, 1961), it is probably that the one trace condition is derived from the three trace condition by fusion of the three traces, while the five trace condition is derived from the three trace condition by the division of the lateral bundles.

The bundles are collateral in structure. Solereder (1908) reported that in P. incana the outer bundles are larger in size, whereas in P. brachyphylla, the inner bundles are larger. Plate 10A shows the union of the two bundles. Therefore, the size of the bundles depends upon the proximity of the place samples to the site of fusion or division of bundles. An endodermis is commonly found aroung the vascular bundles of some plants (Solereder, 1908). Johnson (1914) reported the presence of an endodermis in P. hispidula. In the Hawaiian species studied by Yuncker and Gray (1934) no endodermis was observed; instead closely packed parenchyma cells surrounded the vascular bundles. In agreement with Yuncker and Gray (1934), no endodermis around the bundles of mature stems in any species studied at present was found. However, around each bundle there is a layer of parenchyma cells which have thinner walls and are of smaller size than the ground parenchyma cells (Plate 10C). In young stems, (Plate 10D) the layer contains starch, thus forming a starch sheath. The endodermis would occur in this position if it developed. Therefore, the starch sheath is considered homologous with the endodermis (Guttenberg, 1943). Taking this consideration into account, it is interpreted here that the development of the endodermis in the Hawaiian species has been reduced, leaving a layer of parenchyma cells around each vascular bundle. The phloem consists of large sieve tube elements with prominent companion cells. In P. variegata, some phloem elements are collenchymatous (Solereder, 1908). No collenchymatous cells were found in

the phloem region of the Hawaiian species studied by me. In P. hesperomannii, P. hirtipetiola, P. kokeana, and P. lilifolia, a group of unlignified parenchyma cells was found in the outermost part of the phloem (Plate 10C), forming a bundle cap. These cells were not found in the other Hawaiian species studied. According to Hofstadt (1914) the lignified pericycle of the related genus Piper occupies the same position as these parenchyma cells. He suggested that this group of cells demonstrated the breaking up of the pericycle in the process of the acquisition of the herbaceous habit. The genus Peperomia, as represented by the Hawaiian species, has shown further reduction by eliminating the lignification of these cells. Lack of these parenchyma cells in the rest of the Hawaiian species demonstrated further reduction from the preceding condition. Unlignified parenchyma cells and lignified vessel elements comprise the xylem. No tracheids were found. In some species such as in P. lilifolia the number of vessels is low (7 vessels per vascular bundle); in others, e.g., P. oahuensis, it is high (20 vessels per vascular bundles). Johnson (1914) believed that the poor development of the vessels in P. pellucida was related to its moist habitat. Comparing this species with the xerophytic species such as P. reflexa, he showed a difference in the development of the bundles. The bundles were more highly developed in the latter species. The present investigation does not support Johnson's (1914) view. P. oahuensis, P. dextrolaeva, and P. koolauana, which grow in more moist conditions than

P. reflexa, P. leptostachya, and P. cookiana develop as many or more vascular bundles than the latter species. Also from the number of vessels in cross sections of each vascular bundle, it is shown that some of the species growing in moist conditions develop more vessels than the species of the arid habitats. The thickenings of the vessel walls are usually scalariform, although annular and spiral thickenings are not uncommon. The perforation plates are always simple (Plate 10F). Both oblique and transverse end wall inclinations are found in the Hawaiian species. Johnson (1914), Yuncker and Gray (1934), and Skottsberg (1948) reported that no cambium was found in the species they studied. In the present studies cambium development was not observed in some Hawaiian species such as P. alternifolia, P. cookiana, P. dextrolaeva, P. eekana, P. ellipticibacca, P. latifolia, P. leptostachya, P. oahuensis, P. reflexa, P. sandwicensis, and P. waikamoiana. However, in the largest stems of P. hesperomannii, P. hirtipetiola, P. lilifolia, P. kokeana, and P. subpetiolata the phloem and the xylem tissues are separated by small parenchyma cells which possibly represent a remainder of a pro-cambium layer (Plate 10E). Although the sections were cut from the largest stems a developing vessel member can be identified. Secondary growth as a whole has been greatly reduced.

GENERAL DISCUSSION AND CONCLUSIONS

The carpel morphology, meiotic chromosome number, meiotic irregularities, leaf arrangement, and the anatomy of leaf and stem of 27 Hawaiian species of Peperomia have been studied.

The family Piperaceae, to which Peperomia belongs, is included among the putative primitive angiosperms (Thorne, 1966). The two largest genera in the family Piperaceae are Peperomia and Piper. There is no agreement as yet which of the two genera is more advanced than the other. In listing primitive vs. advanced characters in angiosperms, Smith (1966) considered the woody habit, the cylindric arrangement of vascular bundles, and the presence of stipules as among the primitive characters. The genus Peperomia, at least as shown by the Hawaiian species, differs from the genus Piper in having scattered vascular bundles, no stipules and its herbaceous habit. Vascular cambium was not observed in most species studied. Consequently, no secondary growth was observed in these species. In some species, a residual vascular cambium was observed. The xylem components of Peperomia differs from those of Piper in lacking fiber elements; thus, it consists of only lignified scalariform vessels and unlignified parenchyma cells. The family Piperaceae is characterized by simple perforation plates in the vessels (Metcalfe and Chalk, 1950). However, Solereder (1908) and Record (in

Metcalfe and Chalk, 1950) pointed out the occurrence of scalariform plates with few bars in Piper. None of the Hawaiian species of Peperomia observed by me has scalariform perforation plates, but only simple ones. Simple perforation plates are considered to be more advanced than scalariform plates. In discussing the anatomy of Piper methysticum and P. umbellatum, Hofstadt (1914) concluded that the herbaceous habit has evolved from the woody one through the elimination of secondary growth. Among the characters listed by him as evidences in the process of elimination of secondary growth, there are: breaking up of the pericycle and its confinement to only regions outside of each vascular bundle, and the scattering of the bundles in the pith. No pericycle was observed in the Hawaiian species studied at present, but a group of small parenchyma cells was found outside of each vascular bundle, thus, in the position of pericycle. In all these species the vascular bundles are scattered in the pith. Metcalfe and Chalk (1950) pointed out that endodermis is developed to varying degrees in Piper. Johnson (1914) reported the occurrence of endodermis in Peperomia hispidula. The development of the endodermis was not observed in the species studied in this thesis. In young stems, however, starch grains are accummulated in these cells, forming a starch sheath around each bundle. Since this starch sheath occupies the position of the endodermis, it is considered homologous with the endodermis (Guttenberg, 1943). It is interpreted here that there is a reduction of the development of the Casparian strip in these cells. The present investigation

supports Rousseau's (1927) view in concluding that Peperomia has undergone major modification from the type of Piper. The present study provides evidence that in Peperomia secondary growth is eliminated by reduction of cambial activity. The absence of fibers in the xylem of Peperomia may be interpreted as a reduction from the Piper type through the elimination of fibers from the xylem elements.

Unlike the carpels of all other genera in the family Piperaceae, the genus Peperomia is characterized by having one single stigma, although it may be cleft giving an impression of two stigmas (Trelease and Yuncker, 1950). In studying carpel vascularization in the species of Hawaiiana Peperomia, it is concluded here that the presence of three dorsal carpellary bundles and three stigmas indicates a primitive condition of three fused carpels in the genus. Such a condition is found in P. erythroclada, P. hirtipetiola, and P. lilifolia. The reduction of the three carpellate gynoecium to a unicarpellate gynoecium found in P. membrancea, P. cookiana, etc., is demonstrated by a reduction in the number of stigmas accompanied by a reduction in the number of dorsal carpellary bundles. Therefore, the Hawaiian species provide evidence for interpreting the carpel of Peperomia as derived by reduction from the type of Piper, whose gynoecium consists of three carpels (Johnson, 1914). A true monomerous gynoecium is shown in some species such as P. reflexa, P. cookiana, etc.

Yuncker (1933) proposed the subgenus Hawaiiana for a group of Hawaiian

species whose stigma is divided and located apically or slightly subapically, to be distinguished from the subgenus Sphaerocarpidium whose stigma is single or divided and located subapically. The difference between the apical and subapical position of the stigmas is hardly distinguishable. Among the Hawaiian species assigned to the subgenus Sphaerocarpidium by Yuncker (1933) only P. cookiana, P. leptostachya, P. membranacea, P. koolauana, P. kokeana, P. remyi, and P. waikamoiana show the subapical position of the stigma. It is difficult to tell whether the stigmas of the remaining species of the subgenus Sphaerocarpidium studied here are located apically or subapically. Moreover, there is a variation in the number of stigmas and its corresponding number of dorsal carpellary bundles in these species. Only one dorsal carpellary bundle was found in the ovary wall of P. cookiana, P. leptostachya, etc., whereas both one and two dorsal carpellary bundles accompanied by one or two stigmas were found in the remaining species of the subgenus Sphaerocarpidium. Difficulty in determining the position of the stigma was also found in the species assigned to the subgenus Hawaiiana. P. eekana, P. hypoleuca, P. expallescens, and P. hawaiiensis were described as having apical and slightly subapical stigmas (Yuncker, 1933). In comparing these species with some species of the subgenus Sphaerocarpidium such as P. sandwicensis, the difference in stigma position becomes obscure. The occurrence of one or two vascular bundles in the ovary walls gives further evidence of the similarity

between the two groups. Therefore, it is concluded here that the study of the vascularization of the gynoecium and the number of stigmas on the gynoecium does not support Yuncker's (1933) grouping of the Hawaiian species into their corresponding subgenera.

Of the 20 different Hawaiian species investigated cytologically in this study, almost all show multiples of 11 chromosomes in their gametic cells. Four of them, namely P. cookiana, P. koolauana, P. kokeana, and P. membranacea contain n = 44 and 48; the remaining species have n = 22, except P. linifolia which has a variation of n = 24. In comparing the chromosome number of the Hawaiian species with those species reported by the previous workers (Abele, 1923; Blot, 1960; Hauser, 1918; Johnson, 1914; Martinoli, 1948; Sugiura, 1936), the Hawaiian species show the tendency of having twice as many chromosomes. Meiotic irregularities are commonly observed in species having n = 44 and 48, whereas among species with n = 22 only some show such irregularities. These meiotic irregularities resulted in pollen sterility. On the basis of available data Sharma and Bhattacharyya (1959) concluded that all species of Peperomia excepting P. sintenisii represent a single evolutionary series starting from 11 chromosomes in the basic set. The present study supports the conclusion drawn by Sharma and Bhattacharyya (1959). None of the Hawaiian species observed in the present studies as well as most of those reported by Skottsberg (1955) has other than n = 22 in the basic set. Of the species with n = 44 and 48, six species have one carpel and their stigmas are

located subapically. Thus, there is strong correlation between the degree of polyploidy, the number of carpels, and the position of stigmas on the carpel. Therefore, it is concluded that the subapical position is a derived condition since polyploidy is a derived one. The difference in chromosome counts between the two collections of P. lilifolia from Mt. Kaala (Oahu), and Waikamoi (Maui) suggests the possible occurrence of chromosomal biotypes in the Hawaiian species of Peperomia. That speciation in Hawaiian Peperomia may possibly be achieved through variations of somatic chromosome complements as described by Sharma and Mazumdar (1956) is shown from the difference between the somatic chromosome counts (Skottsberg, 1955) and the gametic chromosome numbers reported in this paper.

The study of the arrangement of leaf primordia supports Murty's (1959) interpretation that the whorled leaf arrangement in P. reflexa has been derived from a decussate-opposite leaf arrangements. The remaining species of Hawaiian Peperomia show spiral (P. lilifolia type) or subopposite (P. alternifolia type) arrangement of primordia. From the studies of the shoot apex and the nodal anatomy, it is concluded here that the whorled arrangement in the Hawaiian species are caused by: (1) the attachment of the primordia to their lateral neighbors, (2) the reduction of internode elongation due to reduction of both cell division and cell elongation, and (3) the fusion of vascular bundles belonging to two neighboring leaf primordia. There is a suggestion that the whorled arrangement found in the mature stem is genetically fixed,

because these species having alternate leaf arrangement in the mature stem such as in P. oahuensis, never produce whorled leaves on their stems.

It seems there is no correlation between the specialization of the carpel and the arrangement of leaves on the stem. The whorled arrangement may be found in both species with specialized carpels (P. cookiana type) and in those with less specialized carpels (P. hirtipetiola type), whereas the alternate arrangement is confined to the species having carpels with one or two stigmas accompanied by one or two dorsal carpellary bundles. Some of the species with one or two stigmas may have a whorled leaf arrangement, e.g., P. ellipticibacca. Therefore, it is suggested here that the mode of leaf arrangement and the degree of carpel specialization are independent of each other.

As the purpose of the investigation was to evaluate the usefulness of certain criteria -- carpel morphology, meiotic behavior, mode of leaf arrangement, and leaf and stem anatomy -- as a basis for phylogenetic interpretation, it can now be said for the Hawaiian Peperomia species, that:

1. The primitive condition of the gynoecium may have been tricarpellary and that the present day species show all stages in reduction to two carpels and finally to one carpel.

2. The basic chromosome numbers of the Hawaiian species are n = 11 and n = 12. On the basis of available data, it can be suggested that numerical

alteration of chromosomes, polyploidy, and hybridization have played important roles in the evolution of the Hawaiian species.

3. The whorled arrangement of leaves in Peperomia species has been derived from a spiral or rarley a decussate-opposite leaf arrangement. This type of arrangement although it can be explained developmentally, has the tendency to be genetically fixed but has developed independently of the other characters investigated.

4. No characters of leaf anatomy could be correlated with advanced or primitive characters shown by other organs. The herbaceous habit in Peperomia has been acquired through the reduction of cambial development, endodermis, pericycle, and xylem fibers.

Further substantiation using species collected from different parts of the world would be necessary in order to validate the suggestions outlined in this paper for the genus as a whole.

SUMMARY

Carpel morphology, meiotic chromosome number, meiotic irregularities, leaf arrangement, and the anatomy of leaf and stem of 27 Hawaiian Peperomia species representing three subgenera have been studied, resulting in the following observations:

1. The flowers of the Hawaiian Peperomia species have one, two, or three stigmas.

2. There are one, two, and three dorsal carpellary bundles in the ovary wall.

3. The number of stigmas and dorsal carpellary bundles show that the gynoecium is either monocarpellary, bicarpellary, or tricarpellary. By reduction, a tricarpellary condition has given rise to a bicarpellary, with a monocarpellary gynoecium being the ultimate product.

4. The bending of the ovular trace alone cannot be used to interpret the position of the ovule as lateral.

5. Gametic chromosome numbers of the Hawaiian species studied here are 22 or multiples of 22. One species exhibits a chromosomal biotype of n = 24.

6. Some species show meiotic irregularities such as precocious disjunction, unpaired chromosome at M I, lagging chromosomes, formation

of bridges, micronuclei formation in the cells of tetrads and abnormal contraction of chromosomes.

7. The presence of n = 22 and its multiple, and the meiotic irregularities are taken as indications that hybridization has played a major role in the formation of species in the genus.

8. The possible role of alteration of chromosome number in somatic cells, and polyploidy, in the speciation of the genus is discussed.

9. No leaf arrangement other than spiral is found in the shoot apex, except in P. reflexa which has an opposite-decussate leaf primordia. These conditions have given rise to other leaf arrangement found on mature stems.

10. With regard to neighboring cells, the Hawaiian species show three stomatal types, i.e., a type with numerous subsidiary cells, an anisocytic type, and a tetracytic type.

11. The presence of residual vascular cambium, pericycle, and endodermis were observed in some species.

12. The loss of cambial activity, and a reduction in the development of pericycle and endodermis, as well as the lack of xylem fibers are interpreted as degrees of advancement toward simplification from the related genus Piper.

13. This study does not support Yuncker's grouping of the Hawaiian species into subgenera.

TABLE 1. DISTRIBUTION OF SPECIES OF PEPEROMIA IN THE HAWAIIAN ISLANDS (ST. JOHN, 1943; YUNCKER, 1933, 1937; 1949).

SPECIES	KAUAI	OAHU	MOLOKAI	MAUI	HAWAII
P. alternifolia			x		
cronwelliae					x
cookiana	x		x	x	x
cornifolia					x
degeneri			x		
dextrolaeva		x			
eekana				x	
ellipticibacca		x			
erythroclada				x	
expallescens			x	x	x
fauriei			x		
forbessii			x		
globulanthera				x	
haupuensis	x				
hawaiiensis					x
helleri	x		x		
hesperomanni	x				
hirtipetiola				x	
hypoleuca		x			x
kalihiana		x			
kokeana	x				
koolauana		x			
kulensis				x	
latifolia	x	x	x	x	x
leptostachya	x	x	x	x	x
ligustrina				x	
lilifolia		x	x	x	x

TABLE 1. (continued) DISTRIBUTION OF SPECIES OF PEPEROMIA IN THE HAWAIIAN ISLANDS (ST. JOHN, 1943; YUNCKER, 1933; 1937; 1949).

SPECIES	KAUAI	OAHU	MOLOKAI	MAUI	HAWAII
P. macraeana				x	
mapulehuana			x		
mauiensis			x	x	
maunakeana					x
membranacea		x			
oahuensis	x	x			
pellucida		x			
pololuana					x
reflexa	x	x	x	x	x
remyi	x	x	x	x	x
rigidolimba					x
rockii			x		
sandwicensis	x	x	x	x	
subpetiolata				x	
treleasi				x	
trichostigma				x	
waikamoiana				x	
waipioana					x
	11	13	17	19	16

TABLE 2. PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
+* P. alternifolia	44	Molokai	T. Newell
	47	Molokai	T. Newell
+* P. cookiana	46	Molokai	T. Newell
	48	Molokai	T. Newell
	103	Hawaii	S. Notoatmodjo
	104	Hawaii	S. Notoatmodjo
	117	Hawaii	K. Kartawinata
	118	Hawaii	E. Bishop
	141	Maui	S. Notoatmodjo
	181	Maui	C. Smith
	234	Hawaii	S. Notoatmodjo
	219	Hawaii	S. Notoatmodjo
	240	Hawaii	K. Nagata
+* P. dextrolaeva	72	Oahu	J. Obata
	89	Oahu	J. Obata
*P. eekana	132	Maui	W.Hoe
	142	Maui	S. Notoatmodjo
	189	Maui	C. Smith
+* P. ellipticibacca	1	Oahu	H. Whittier
	140	Maui	S. Notoatmodjo
	242	Oahu	D. Herbst
* P. erythroclada	210	Hawaii	P. Gehring
	231	Hawaii	E. Bishop
* P. expallescens	50	Maui	T. Newell
	57	Maui	T. Newell
	185	Maui	C. Smith
	188	Maui	C. Smith
+* P. globulanthera	194	Maui	C. Smith
	205	Maui	C. Smith

TABLE 2. (continued) PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
+* P. hawaiiensis	198	Hawaii	P. Gehring
	201	Hawaii	P. Gehring
	218	Hawaii	S. Notoatmodjo
	220	Hawaii	S. Notoatmodjo
+* P. hesperomannii	6	Kauai	H. Whittier
	8	Kauai	H. Whittier
	12	Kauai	H. Whittier
	31	Kauai	E. Bishop
	38	Kauai	C. Lamoureux
	41	Kauai	S. Nicharat
	42	Kauai	S. Nicharat
	68	Kauai	C. Lamoureux
	167	Kauai	E. Bishop
	243	Kauai	S.Notoatmodjo
*P. hirtipetiolata	52	Maui	T. Newell
	53	Maui	T. Newell
	135	Maui	S. Notoatmodjo
+* P.hypoleuca	13	Hawaii	C. Lamoureux
	65	Hawaii	M. Doty
	66	Hawaii	M. Doty
	176	Hawaii	T. de Aussen
	221	Hawaii	S. Notoatmodjo
	223	Hawaii	S. Notoatmodjo
	237	Hawaii	E. Bishop
*P. kalihiana	27	Oahu	J. Obata
+* P. kokeana	5	Kauai	H. Whittier
	244	Kauai	S. Notoatmodjo
+* P. koolauana	4	Oahu	H. Whittier
	28	Oahu	J. Obata
	78	Oahu	J. Obata
	86	Oahu	J. Obata
	87	Oahu	J. Obata
	91	Oahu	J.Obata

TABLE 2. (continued) PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
+* P. latifolia	9	Oahu	S. Notoatmodjo
	39	Oahu	S. Notoatmodjo
	60	Oahu	T. Newell
	62	Oahu	T. Newell
	96	Oahu	S. Notoatmodjo
	116	Oahu	S. Notoatmodjo
	124	Oahu	S. Notoatmodjo
	166	Oahu	S. Notoatmodjo
	165	Oahu	K. Kartawinata
	163	Oahu	K. Kartawinata
+* P. leptostachya	20	Oahu	J. Obata
	43	Kauai	S. Nicharat
	58	Molokai	P. Trask
	59	Molokai	P. Trask
	67	Hawaii	C. Lamoureux
	69	Oahu	E. Shimizu
	75	Oahu	T. Green
	79	Oahu	J. Obata
	145	Oahu	B. Long
	146	Oahu	K. Kartawinata
	156	Oahu	S. Notoatmodjo
	159	Oahu	S. Notoatmodjo
	160	Kauai	B.Long
	175	Hawaii	T. de Aussen
	226	Hawaii	S. Notoatmodjo
	228	Hawaii	E. Bishop
	246	Kauai	S. Notoatmodjo
+* P. lilifolia	2	Oahu	H. Whittier
	3	Oahu	H. Whittier
	100	Oahu	S. Nicharat
	109	Oahu	S. Notoatmodjo
	116	Oahu	S. Notoatmodjo
	138	Kauai	S. Notoatmodjo
	172	Hawaii	S. Notoatmodjo
	179	Oahu	S. Notoatmodjo
	183	Maui	C. Smith
	235	Hawaii	E. Bishop

TABLE 2. (continued) PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
*P. macraeana	187	Maui	C. Smith
	204	Maui	P. Gehring
	209	Maui	P. Gehring
	215	Hawaii	S.Notoatmodjo
	225	Hawaii	S.Notoatmodjo
*P. membranacea	32	Oahu	S. Notoatmodjo
	33	Oahu	S. Notoatmodjo
	63	Oahu	T. Newell
	78	Oahu	J. Obata
	88	Oahu	J. Obata
	92	Oahu	J. Obata
	101	Oahu	S. Notoatmodjo
	108	Oahu	S. Notoatmodjo
	113	Oahu	S. Notoatmodjo
	121	Oahu	A. Young
	127	Oahu	S. Notoatmodjo
+* P. oahuensis	76	Oahu	J. Obata
	98	Oahu	S. Nicharat
	110	Oahu	S. Notoatmodjo
	171	Oahu	S. Notoatmodjo
* P. reflexa	7	Oahu	H. Whittier
	30	Oahu	S. Notoatmodjo
	123	Oahu	A. Young
	154	Oahu	S. Notoatmodjo
+* P. remyi	16	Hawaii	C.Lamoureux
	17	Hawaii	C. Lamoureux
	143	Maui	S. Notoatmodjo
+* P. sandwicensis	29	Oahu	S. Notoatmodjo
	31	Oahu	S. Notaatmodjo
	61	Oahu	T. Newell
	71	Oahu	J. Obata
	80	Oahu	J. Obata
	81	Oahu	J. Obata

TABLE 2. (continued) PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
P. sandwicensis (continued)	82	Oahu	J. Obata
	84	Oahu	J. Obata
	97	Oahu	J. Obata
	147	Oahu	D. Herbst
	152	Oahu	S. Notoatmodjo
	153	Oahu	S. Notoatmodjo
	154	Oahu	S. Notoatmodjo
	158	Oahu	S. Notoatmodjo
	245	Oahu	S. Notoatmodjo
*P. subpetiolata		Maui	T. Newell
P. trichostigma	211	Maui	P . Gehring
	139	Maui	S. Notoatmodjo
+* P. waikamoiana	136	Maui	S. Notoatmodjo
	137	Maui	S. Notoatmodjo
	191	Maui	S. Notoatmodjo
	192	Maui	C. Smith
Unidentified but close to:			
?	56	Hawaii	T. Newell
P. hawaiiensis	197	Hawaii	P. Gehring
P. latifolia	114	Oahu	S. Notoatmodjo
	115	Oahu	S. Notoatmodjo
	122	Oahu	S. Notoatmodjo
	170	Oahu	S. Notoatmodjo
	241	Oahu	S. Notoatmodjo
P. lilifolia	138	Maui	S. Notoatmodjo
P. oahuensis/ P. membranacea	173	Oahu	S. Notoatmodjo

TABLE 2. (continued) PEPEROMIA SPECIES UTILIZED IN THIS STUDY.

SPECIES	COLLECTION NO.	LOCATION	COLLECTORS
Unidentified but close to: (continued)			
P. cookiana	134	Maui	W. Hoe
	227	Hawaii	M. Rajput
	228	Hawaii	E. Bishop

* = Fruiting materials have been studied.

+ = Pollen mother cells have been studied.

TABLE 3. STIGMA NUMBER AND THE CORRESPONDING NUMBER OF DORSAL CARPELLARY BUNDLES.

SPECIES	STIGMA NUMBER	DORSAL CARPELLARY BUNDLE NUMBER
P. alternifolia	one/two	one
cookiana	one	one
dextrolaeva	one/two	one/two
eekana	two	two
ellipticibacca	one/two	one/two
erythroclada	two/three	two/three
expallescens	two	one/two
globulanthera	one/two	one/two
hawaiiensis	one/two	one/two
hesperomannii	one	one/two
hirtipetiola	one/two/three	one/two/three
hypoleuca	two	one/two
kalihiana	two/three	one/two/three
kokeana	one/two	one
koolauana	one/two	one
latifolia	one/two/three	one/two/three
leptostachya	one	one
lilifolia	one/two/three	one/two/three
macraeana	two	two
membranacea	one	one
oahuensis	one/two	one
reflexa	one	one
rigidolimba	two	two
remyi	one	one
subpetiolata	one/two/three	one/two/three
sandwicensis	one/two	one
waikamoiana	one	one

TABLE 4. GAMETIC CHROMOSOME NUMBER OF HAWAIIAN PEPEROMIA SPECIES.

SPECIES	CHR. NO.	LOCATION
P. alternifolia	22	Molokai
cookiana	44	Hawaii
dextrolaeva	48	Oahu
ellipticibacca	22	Oahu
globulanthera	44	Maui
hawaiiensis	22	Hawaii
hesperomannii	22	Hawaii
hypoleuca	22	Hawaii
kokeana	44	Kauai
koolauana	44	Oahu
latifolia	22	Oahu, Kauai
leptostachya	22	Maui
lilifolia	24	Mt. Kaala, Oahu
	22	Maui
membranacea	44	Oahu
oahuensis	22	Oahu
remyi	22	Maui
sandwicensis	22	Oahu
waikamoiana	22	Maui
sample 56	22	Hawaii
sample 122	22	Hawaii
sample 138	24	Maui
sample 197	22	Hawaii

TABLE 5. SOMATIC CHROMOSOME NUMBER OF HAWAIIAN PEPEROMIA SPECIES (AFTER SKOTTSBERG, 1955).

SPECIES	CHR. NO.	LOCATION
P. eekana	c. 36	Maui
erythroclada	28	Maui
expallescens	66	Maui
hawaiiensis	42-46	Hawaii
hesperomannii	48	Kauai
	66	Kauai
lilifolia	c. 42	Hawaii

TABLE 6. IRREGULARITIES IN MEIOSIS OF HAWAIIAN PEPEROMIA SPECIES.

SPECIES	BRIDGES		FRAGMENTS		LAGGING		EARLY SPTION*	
	AI	AII	AI	AII	AI	AII	MI	MII
P. cookiana	+	+	+	+	+	-	-	-
ellipticibacca	+	+	-	+	-	-	-	-
globulanthera	-	-	-	-	-	-	+	-
hawaiiensis	-	-	-	-	+	-	+	+
hypoleuca	-	-	-	-	+	-	+	-
koolauana	-	-	-	-	-	-	-	+
leptostachya	-	-	-	-	-	+	-	-
membranacea	-	-	-	-	-	-	+	-

*SPTION = SEPARATION

TABLE 7. MICROSPORE ABNORMALITY AND POLLEN STERILITY OF HAWAIIAN PEPEROMIA SPECIES.

SPECIES	NO. OF MICRO-NUCLEI	NO. OF POLLEN GRAINS STUDIED	NO. OF GOOD POLLEN	STERILITY
P. cookiana	-	100	77	33%
eekana	-	100	70	30%
expallescens	-	100	84	16%
globulanthera	8	100	83	17%
koolauana	8	100	67	23%
latifolia	-	100	85	15%
leptostachya	8	100	74	26%
lilifolia	-	100	89	11%
membranacea	8	100	82	18%
waikamoiana	10	100	70	30%

TABLE 8. LEAF ARRANGEMENT IN SOME HAWAIIAN PEPEROMIA SPECIES.

SUBGENUS	SPECIES	LEAF ARRANGEMENT	
		YOUNG	MATURE
Micropiper	reflexa	opposite-decussate	whorled
Spaherocarpidium	oahuensis	alternate	alternate
Alternifolia	alternifolia	alternate	alternate
Verticillatae	latifolia	alternate/ sub-opposite	alternate
	membranacea	sub-opposite	whorled
	leptostachya	sub-opposite	whorled
	sandwicensis	alternate	whorled
Hawaiiana	lilifolia	sub-opposite	
	lilifolia	alternate	whorled
	eekana	alternate	whorled
	hypoleuca	sub-opposite	whorled

TABLE 9. THE AVERAGE SIZE AND NUMBER OF CELLS IN EACH TRICHOME OF HAWAIIAN PEPEROMIA SPECIES.

SPECIES	NUMBER OF CELLS	LENGTH IN mm
P. alternifolia	-	-
cookiana	6	0.70
dextrolaeva	-	-
eekana	5	
ellipticibacca	8	0.80
erythroclada	4	0.35
expallascens	7	0.60
globulanthera	-	-
hawaiiensis	-	-
hesperomanni	-	-
hirtipetiola	18	1.70
hypoleuca	10	0.85
kalihiana	5	0.40
kokeana	5	0.30
koolauana	-	-
latifolia	6	0.40
leptostachya	6 and 4	0.40 and 0.2
lilifolia	12	1.0
macraeana	4	0.35
membranacea	-	-
oahuensis	-	-
reflexa	2	1.20
rigidolimba	-	-
remyi	7	5.50
sandwicensis	5	0.40
waikamoiana	8	0.6

TABLE 10. THE NUMBER OF STOMATA PER 100 EPIDERMAL CELLS AND THE AVERAGE LENGTH OF THE GUARD CELLS IN CLOSED STOMATA IN THE ABAXIAL LEAF SURFACE OF HAWAIIAN PEPEROMIA SPECIES.

SPECIES	NUMBER OF CELLS	LENGTH IN μ
P. alternifolia	12	29
cookiana	10	36
dextrolaeva	6	29
eekana	18	40
ellipticibacca	24	33
erythroclada	21	30
expallescens	20	38
globulanthera	20	41
hawaiiensis	11	34
hesperomannii	25	34
hirtipetiola	15	38
hypoleuca	16	31
kalihiana	20	34
kokeana	21	29
koolauana	15	31
latifolia	15	31
leptostachya	12	41
lilifolia	15	38
macraeana	16	31
membranacea	9	41
oahuensis	11	31
reflexa	15	35
remyi	18	41
sandwicensis	16	38
waikamoiana	13	35

Unidentified

TABLE 11. CRYSTAL TYPES IN THE STEMS OF THE HAWAIIAN PEPEROMIA SPECIES.

SPECIES	CRYSTAL TYPES											
	1	2	3	4	5	6	7	8	9	10	11	
P. alternifolia				x					x			
cookiana				x		x			x			
dextrolaeva									x			
eekana				x		x			x			
ellipticibacca									x			
erythroclada				x					x			
expallescens				x	x							
globulanthera			x						x			
hawaiiensis												?
hesperomannii												
hirtipetiola				x		x	x	x	x			
hypoleuca	x			x	x	x		x	x	x	x	
kalihiana					x				x	x		
kokeana									x			
koolauana				x	x	x			x			
latifolia	x	x	x	x	x	x	x	x	x	x	x	
leptostachya		x		x		x			x			
lilifolia	x	x	x						x			
macraeana		x							x			
membranacea				x	x				x			
oahuensis			x						x			
reflexa												?
rigidolumba												?
remyi				x	x				x	x		
sandwicensis	x	x							x			
waikamoiana		x		x							x	

PLATE 1.

A. P. lilifolia. Apical view of cleared flower showing three stigmas. x 60.

B. P. lilifolia. Part of cleared inflorescence. x 20.

a. Bract trace

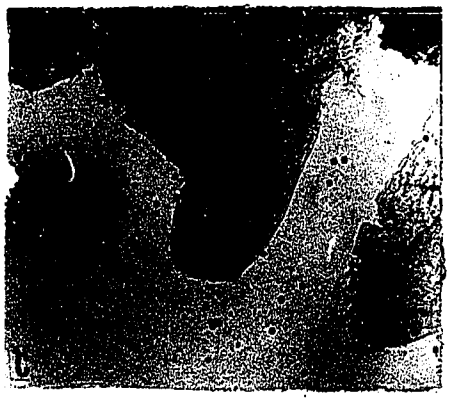
b. Stamen traces

C. P. lilifolia. A cleared stamen. The stamen trace ends in a plate of xylem. x 65.

D. P. lilifolia. Part of cleared inflorescence axis. Two flowers receive traces from a common bundle. x 65.

E. P. leptostachya. Part of cleared inflorescence. The flower trace and the bract trace diverge separately from the main bundle. x 65.


A
B



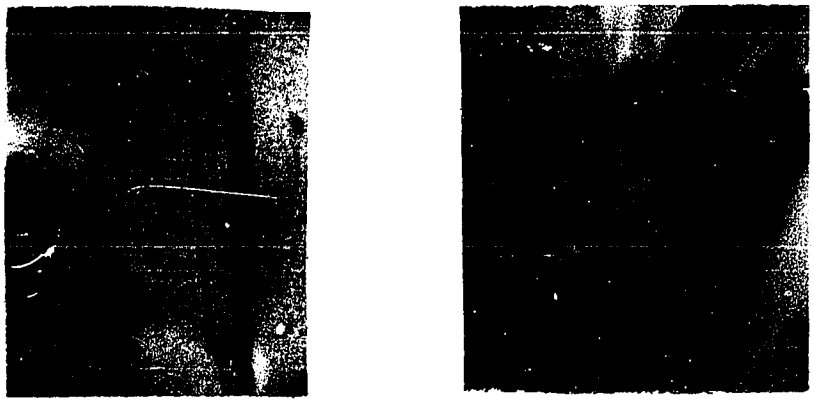


PLATE 2.

A. P. membranacea. A cleared carpel. The dorsal carpellary bundle forms a ring under the stigma. x 66.

B. P. membranacea. A cleared fruit. Plates of tracheary elements were found under the stigma. Note the shrivelled seed. x 70.

C. P. lilifolia. A cleared fruit. The dorsal carpellary bundles protrude into the stigmas. x 70.

D. P. hypoleuca. A cleared fruit. The ovular trace is associated with the adaxial dorsal carpellary bundle. x 70.

E. P. membranacea. Part of cleared inflorescence axis. x 20.

a. Pseudopedicel.

b. Bending of the bract trace.

F. P. membranacea. A cleared mature fruit. "Finger-like projections" have developed above the stigma. x 66.

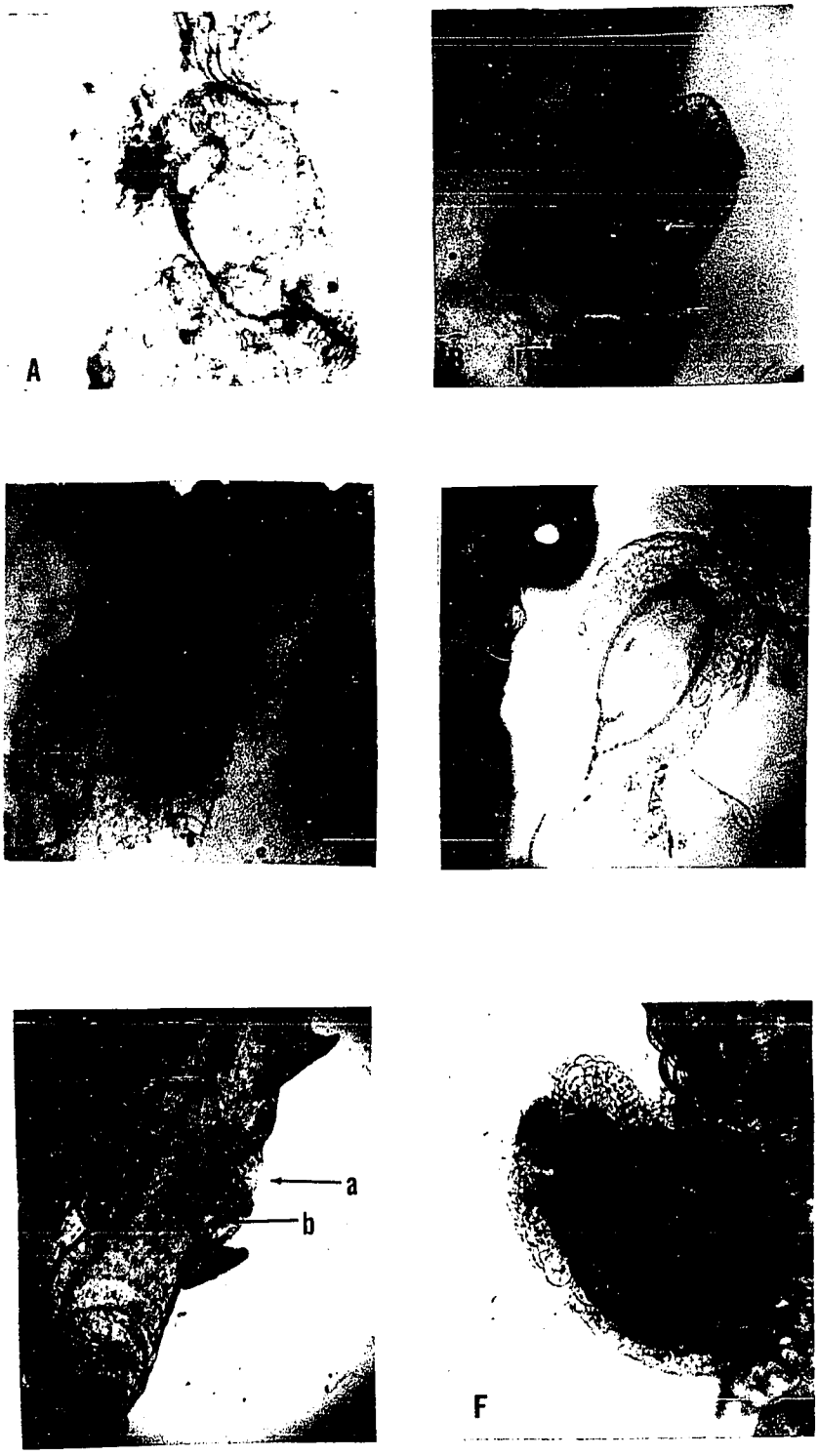

A
a
b
F

PLATE 3.

A. *P. lilifolia.* Metaphase I. x 750.

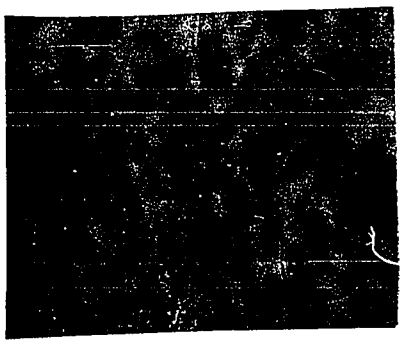
B. Sample no. 227. Prophase I. x 800.

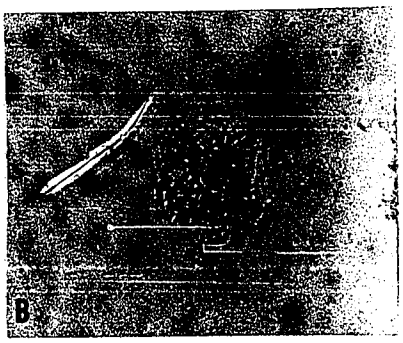
C. *P. cookiana.* Metaphase I. Unpaired chromosomes are quite common. x 750.

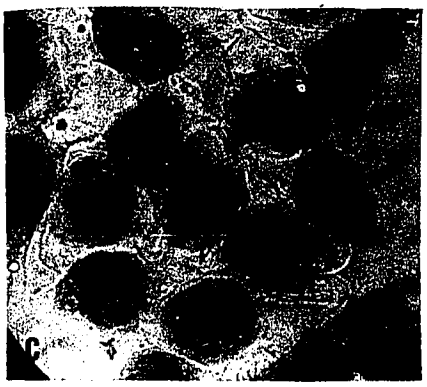
D. *P. cookiana.* Metaphase I, with precocious disjunction. x 800.

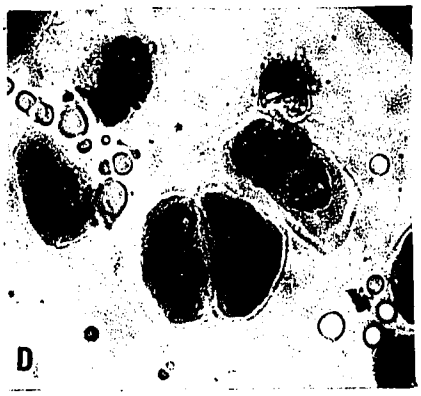
E. *P. hypoleuca* var. *pluvigaudens.* Metaphase II. Abnormal contraction of chromosomes and precocious disjunction are observed. x 750.

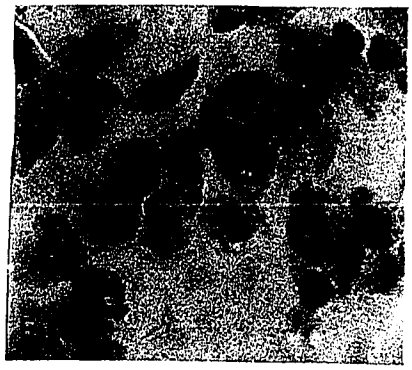
F. *P. cookiana.* Telophase I with bridge and a fragment. x 750.




B


C


D



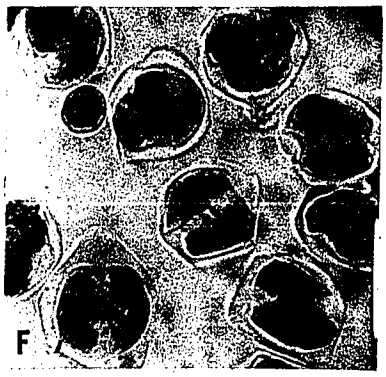

F

PLATE 4.

A. *P. hawaiiensis.* Telophase I. with three poles. x 660.

B. Sample no. 227. Tetrad with polymitotic division. The size of the micronuclei is variable. x 670.

C. *P. globulanthera.* Microspores. One microspore undergoes further division. x 670.

D. *P. alternifolia.* Pollen sterility is indicated by unstained grains. x 640.

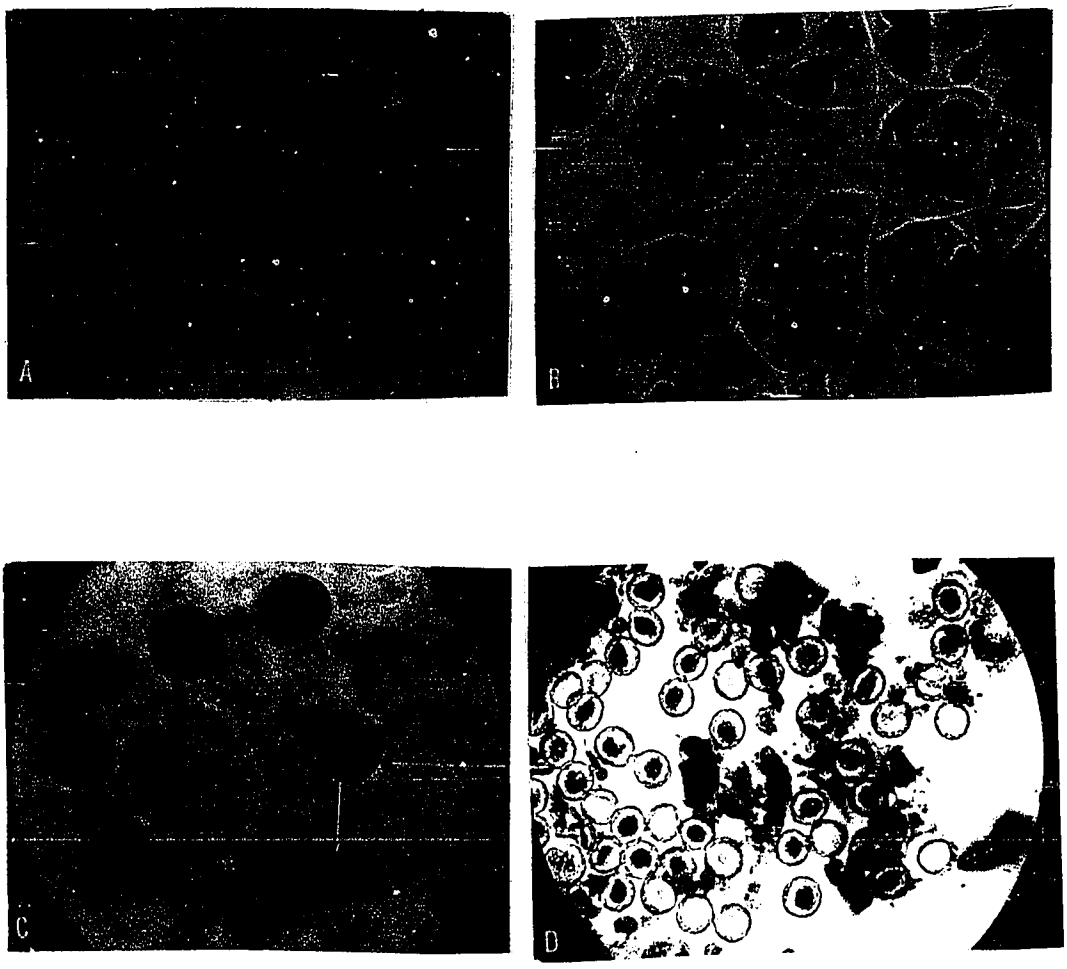

A
B
C
D

PLATE 5.

A. P. hawaiiensis. Involution of young leaf. x 19.

B. P. hypoleuca. Arrangement of primordia in the shoot apex. x 65.

C. P. kokeana. Longitudinal section of fresh stem. The size of pith parenchyma cells is very short in the nodal region. x 20.

D. P. alternifolia. The tip of leaf with trichomes. x 60.

E. P. lilifolia. Trichome with cuticular warts on the surface of the cells. x 225.

F. P. lilifolia. Trichome with membrane at the tip of the apical cell. x 225.

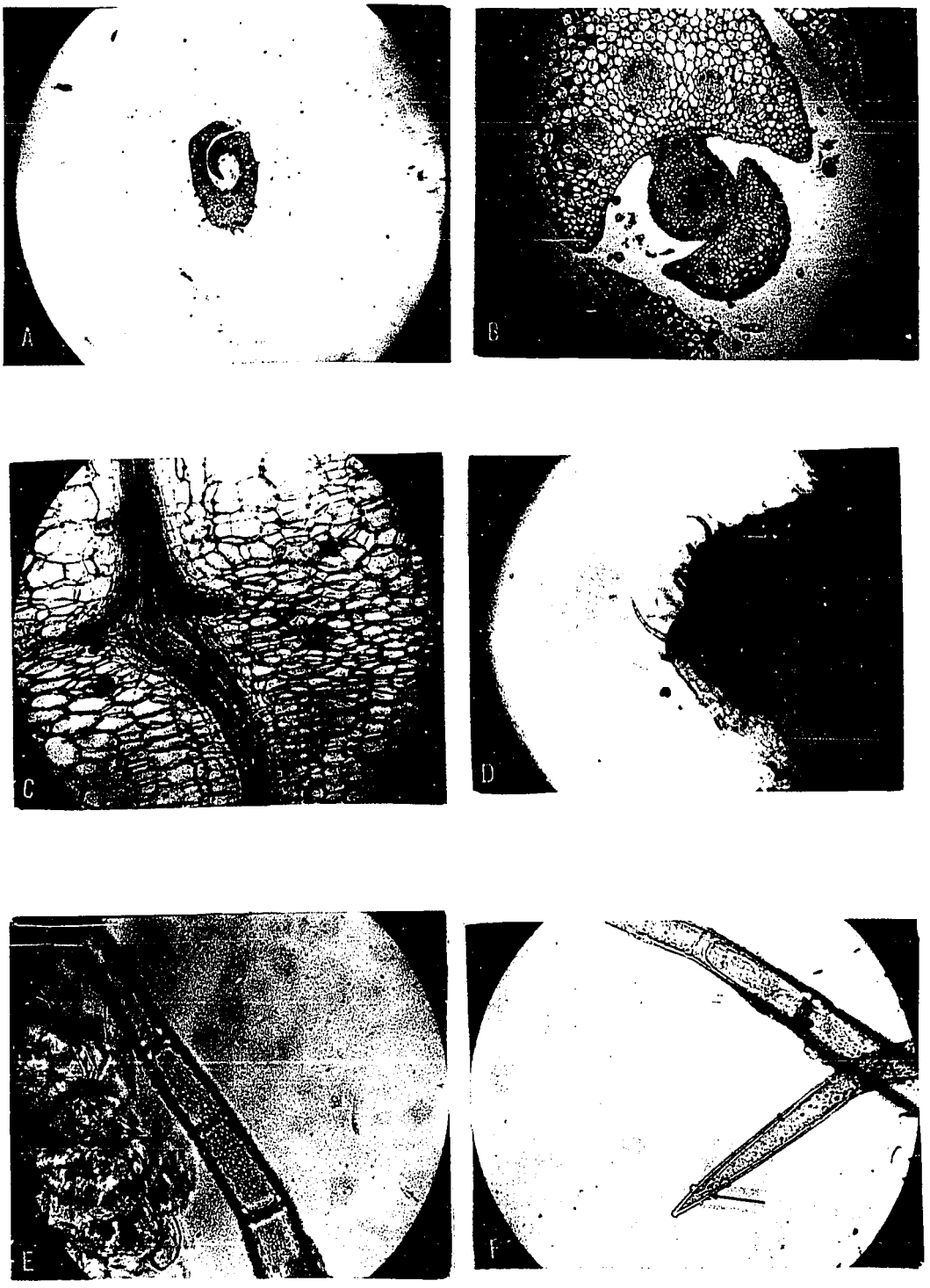

A
B
C
D
E
F

PLATE 6.

A. P. hesperomannii. Transection of leaf epidermis. Projections of cutin on guard cells. x 625.

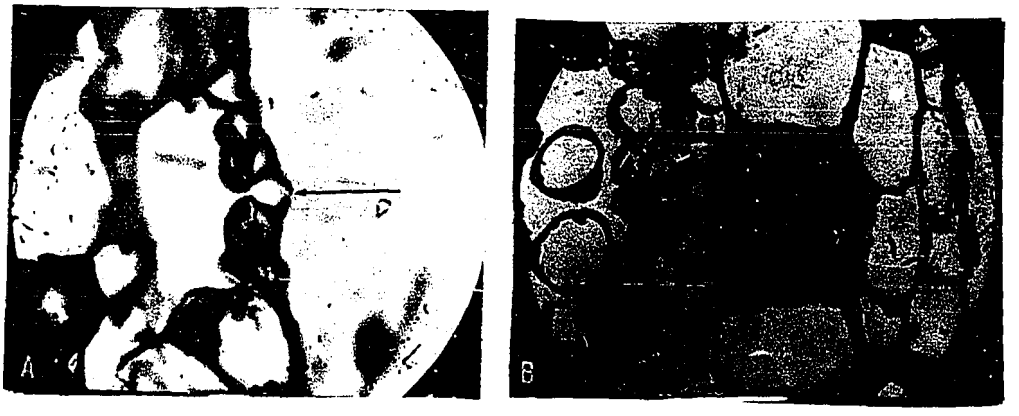
B. P. hypoleuca. Transection of leaf. Pit areas are found in the hypodermal cells. x 250.

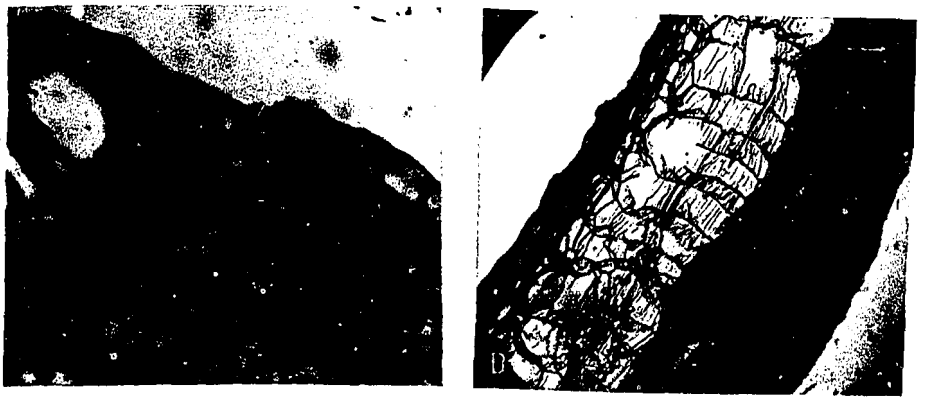
C. P. leptostachya. Transection of leaf. "Cross bands" are found in the hypodermal cells. x 55.

D. P. macraeana. Transection of young leaf. Young oil cells with prominent nuclei are found here. x 670.

E. P. macraeana. Transection of leaf. An oil cell with several compartments of oil droplets. x 800.

F. P. macraeana. Transection of leaf. A mature oil cell with single compartment of oil droplets. The nucleus is disorganized. x 670.


A
B


D


F

PLATE 7.1

A. *P. sandwicensis.* Transection of fresh leaf. A stalk of cellulose attaches the oil basin to the oil cell wall. x 195.

B. *P. cookiana.* Transection of leaf. x 240.

a. Funnel-shaped palisade cell.

b. Collecting cells.

c. Spongy parenchyma.

C. *P. lilifolia.* Transection of midvein. x 66.

D. *P. dextrolaeva.* Transection of midvein. x 66.

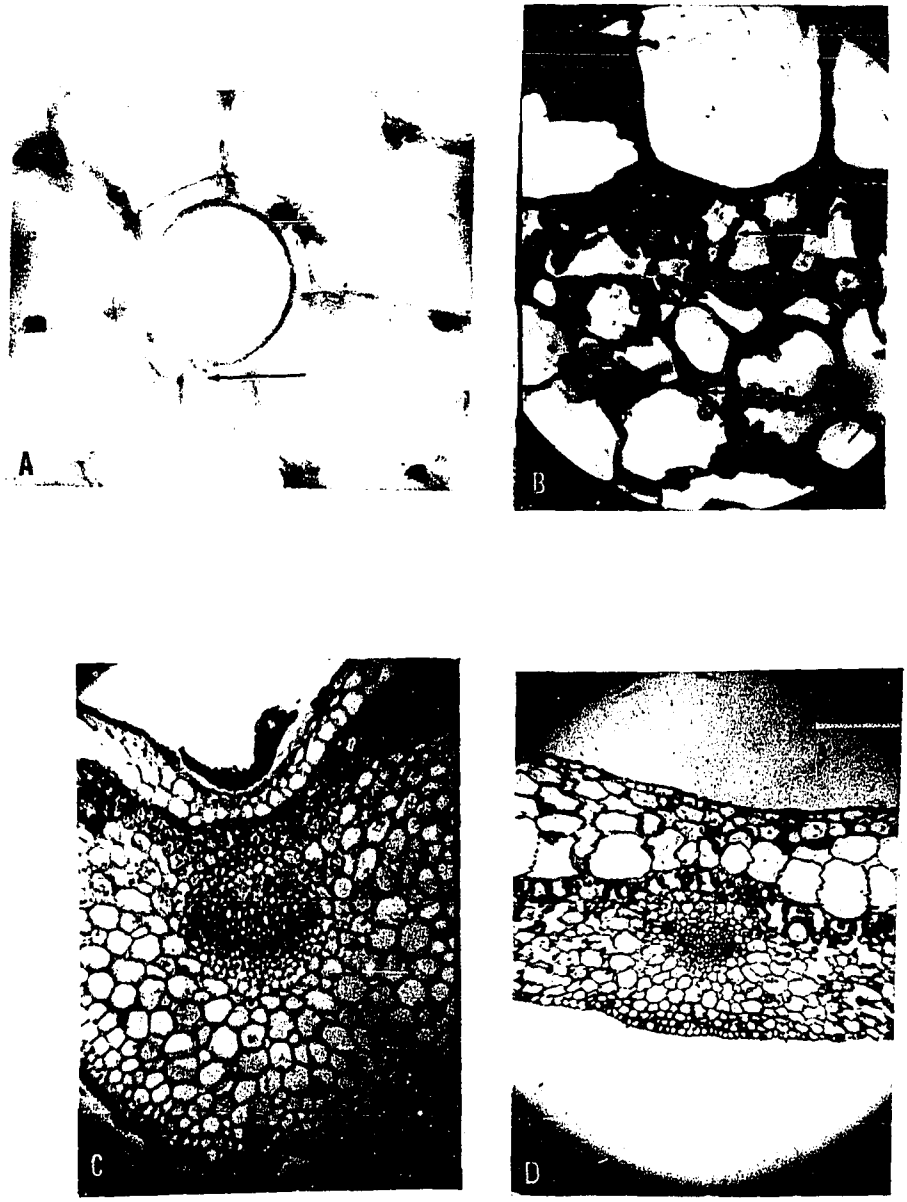

A
B
C
D

PLATE 7.2.

A. *P. sandwicensis.* Transection of petiole. x 18.

B. *P. hawaiiensis.* Transection of petiole. x 18.

C. *P. koolauana.* Transection of petiole. x 18.

D. *P. hesperomanni.* Transection of petiole. x 18.

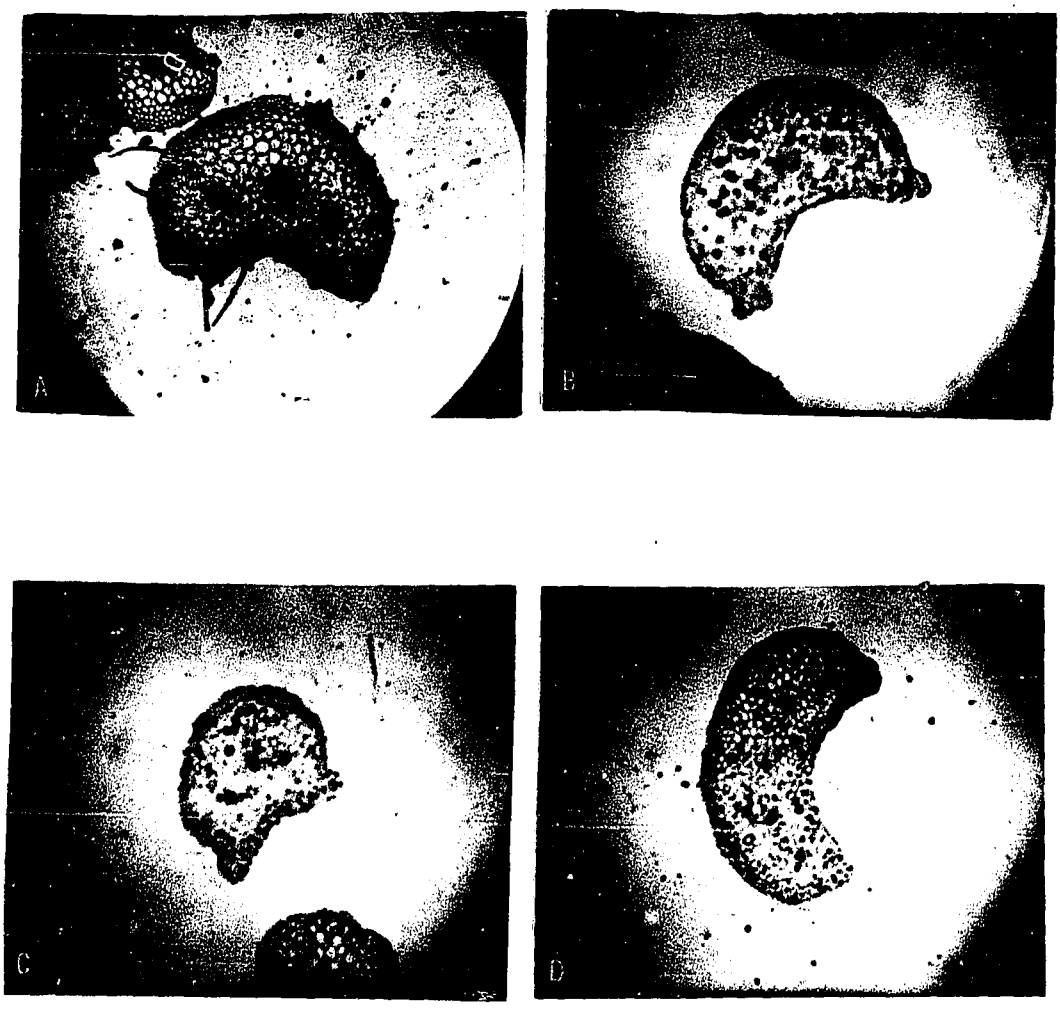

A
B
C
D

PLATE 8.

A. *P. reflexa.* Transection of stem. Note projections of cutin on radial walls of epidermal cells. x 255.

B. *P. reflexa.* Longitudinal section of stem. A glandular trichome was destroyed leaving a depression in the epidermal layer. x 255.

C. *P. leptostachya.* Longitudinal section of stem. Pit areas are found in the walls of collenchyma cells. x 260.

D. *P. hawaiiensis.* Longitudinal section of stem. Pit areas are found in the walls of parenchyma cells. x 260.

E. *P. hawaiiensis.* Longitudinal section of fresh stem showing the pit areas in the walls of parenchyma cells. x 250.

F. *P. latifolia.* Transection of stem. The parenchyma cells have thickened walls. x 250.

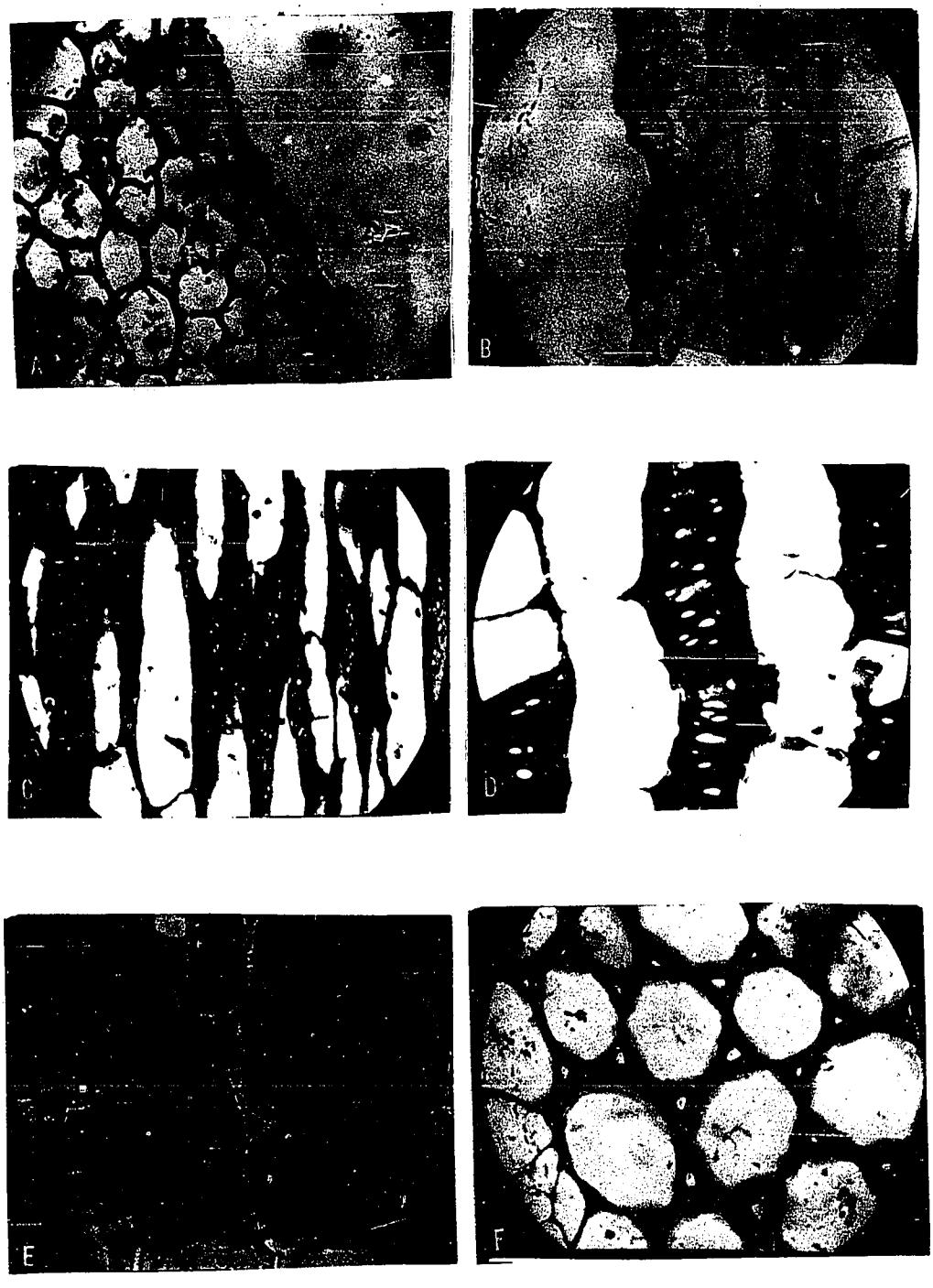

A
B
C
D
E
F

PLATE 9.

A. P. reflexa. Longitudinal section of stem. The distribution of oil cells is associated with vascular bundles. x 66.

B. P. koolauana. Transection of stem. An oil cell resembles in size an ordinary pith cell. x 250.

C. P. reflexa. Transection of stem showing an oil cell with thick wall. x 250.

D. P. hypoleuca. Transection of young stem. Phloem elements have developed before xylem elements. x 250.

E. P. kokeana. Transection of fresh young stem showing the arrangement of vascular bundles. x 27.

F. P. kokeana. Transection of fresh mature stem. x 17.

a. Fusion of two bundles from the same ring.

b. Fusion of two bundles from different rings.

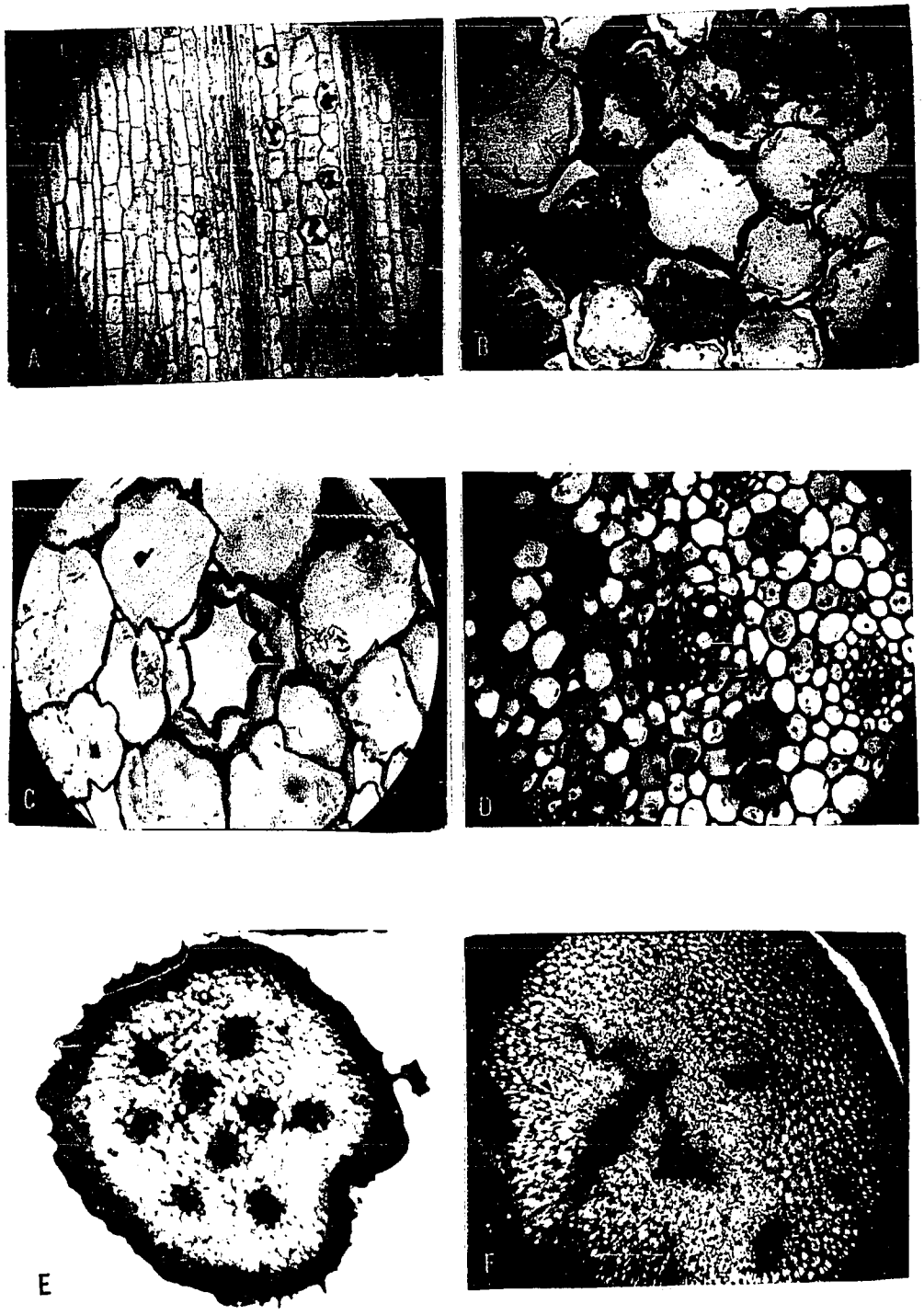

A
B
C
D
E
F

PLATE 10.

A. P. membranacea. A cleared young stem showing the vascularization in the nodal region. x 17.

B. P. kokeana. Longitudinal section of stem. A vascular bundle divides and fuses with another bundle. x 22.

C. P. hesperomanni. Transection of stem. x 66.

a. Small parenchyma cells around the bundle.

b. Undeveloped pericycle.

D. P. kokeana. Transection of young stem stained with IKI. There is a starch sheath around each bundle. x 66.

E. P. hesperomanni. Transection of mature stem. A single vascular bundle is shown with a remainder of pro-cambium. x 260.

F. P. lilifolia. Longitudinal section of stem. A simple perforation plate with transverse end wall is shown. x 250.

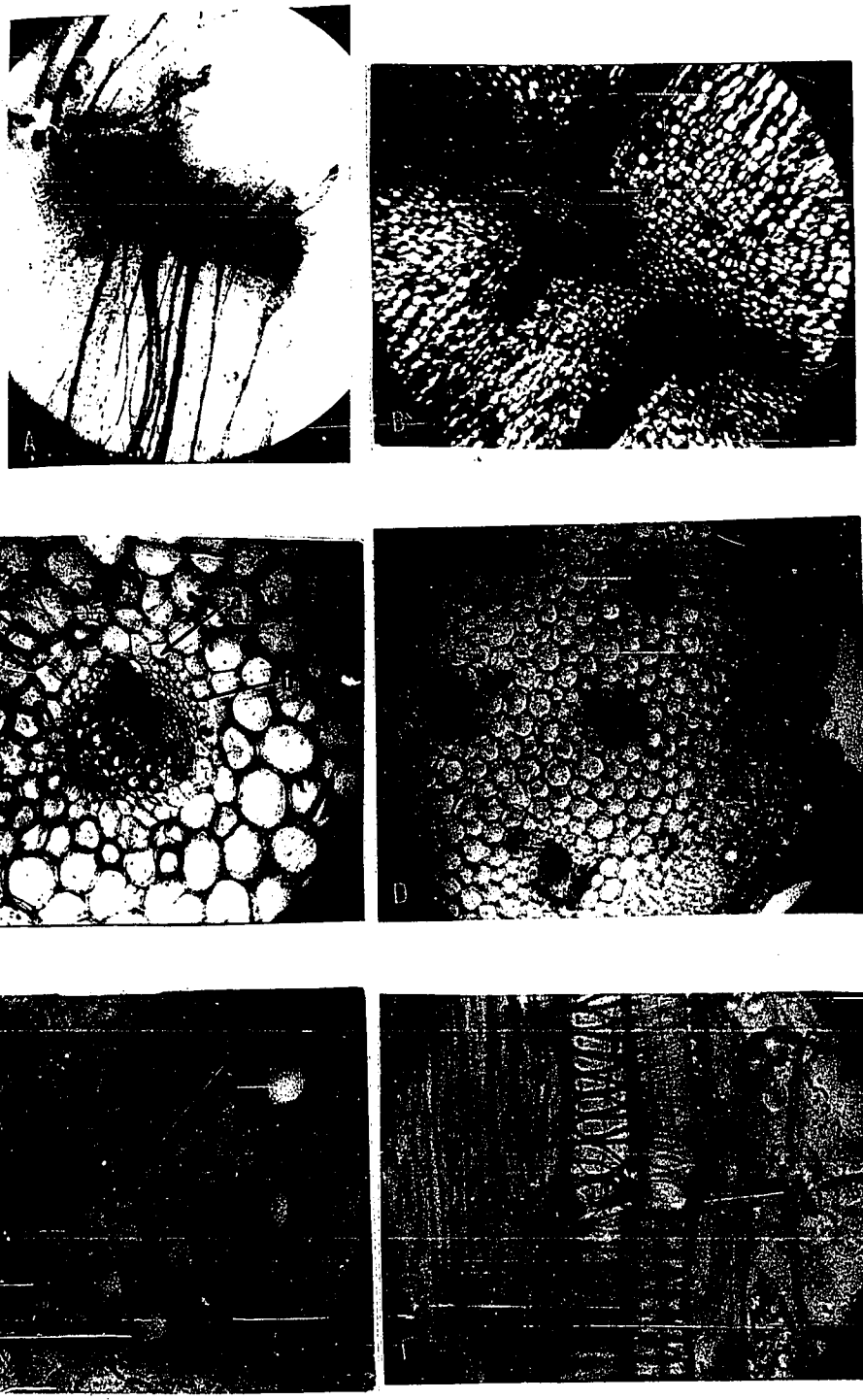

A
B
C
D
E
F

FIGURE 1.

Arrangements of leaf primordia of some Hawaiian Peperomia species:

a. P. reflexa

b. P. oahuensis

c. P. alternifolia

d. P. latifolia

e. P. membranacea

f. P. leptostachya

g. P. sandwicensis

h. P. lilifolia

i. P. eekana

j. P. hypoleuca

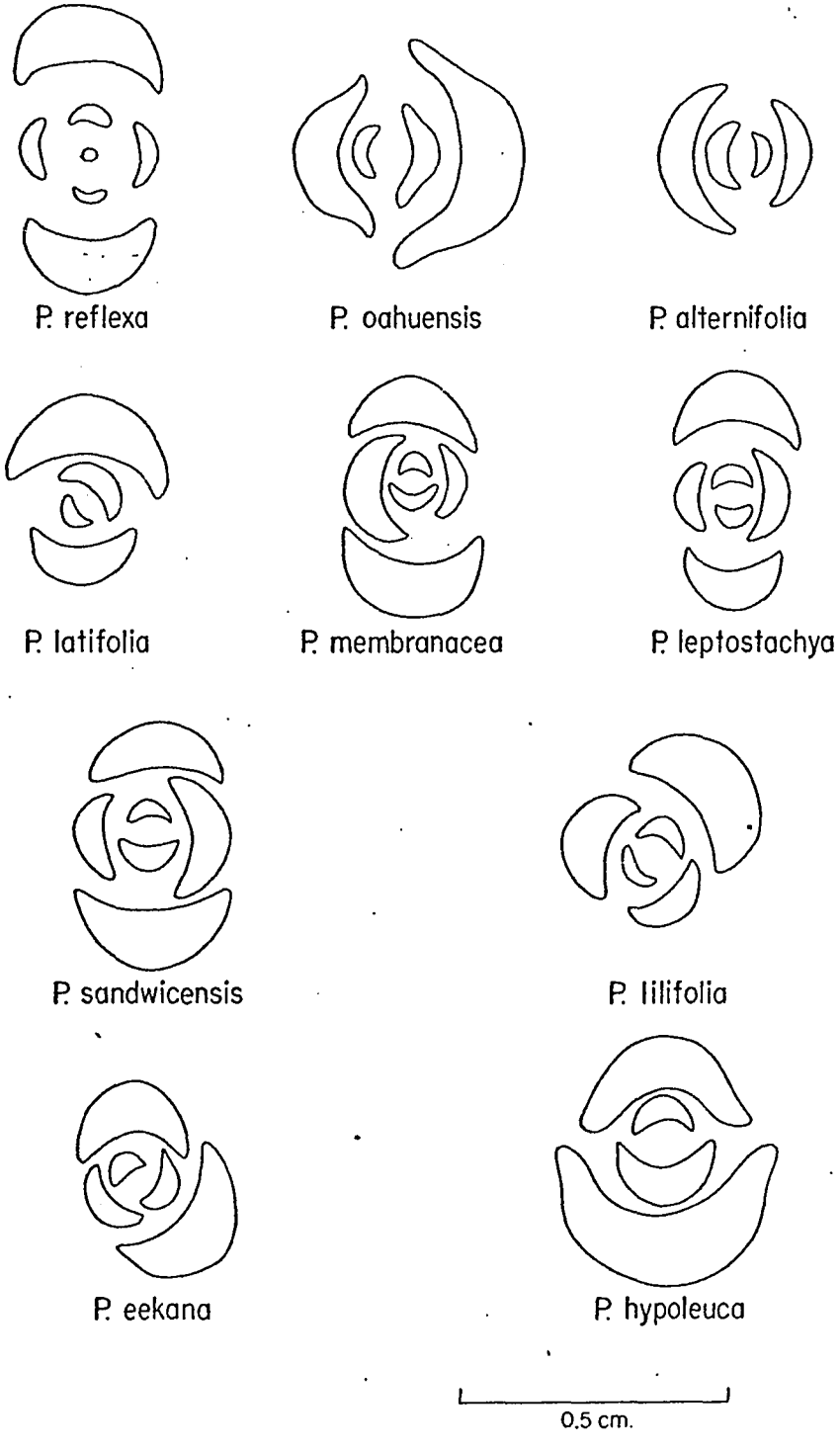

P. reflexa
P. oahuensis
P. alternifolia
P. latifolia
P. membranacea
P. leptostachya
P. sandwicensis
P. lilifolia
P. eekana
P. hypoleuca
0.5 cm.

FIGURE 2.

Transections of nodal region showing the departure of leaf traces.

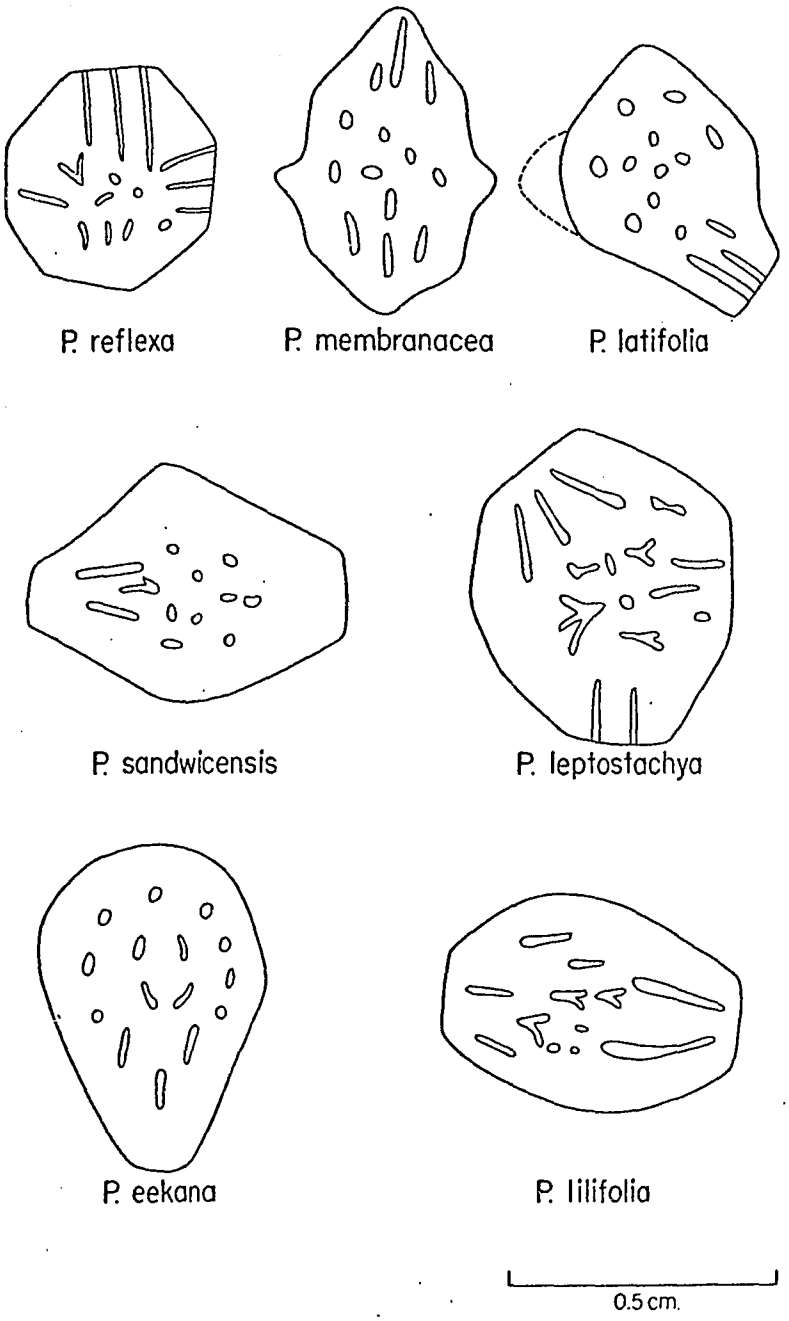

P. reflexa
P. membranacea
P. latifolia
P. sandwicensis
P. leptostachya
P. eekana
P. lilifolia
0.5 cm.

FIGURE 3.

Graphic presentation of leaf length and width from different collections of some Hawaiian Peperomia species. Each dot represents the average size of 11 mature leaves from a single collection.

a. P. alternifolia

b. P. cookiana

c. P. eekana

d. P. hesperomannii

e. P. hirtipetiola

f. P. hypoleuca

g. P. kalihiana

h. P. kokeana

i. P. latifolia

j. P. leptostachya

k. P. lilifolia

l. P. macraeana

m. P. membranacea

n. P. oahuensis

o. P. reflexa

p. P. remyi

q. P. sandwicensis

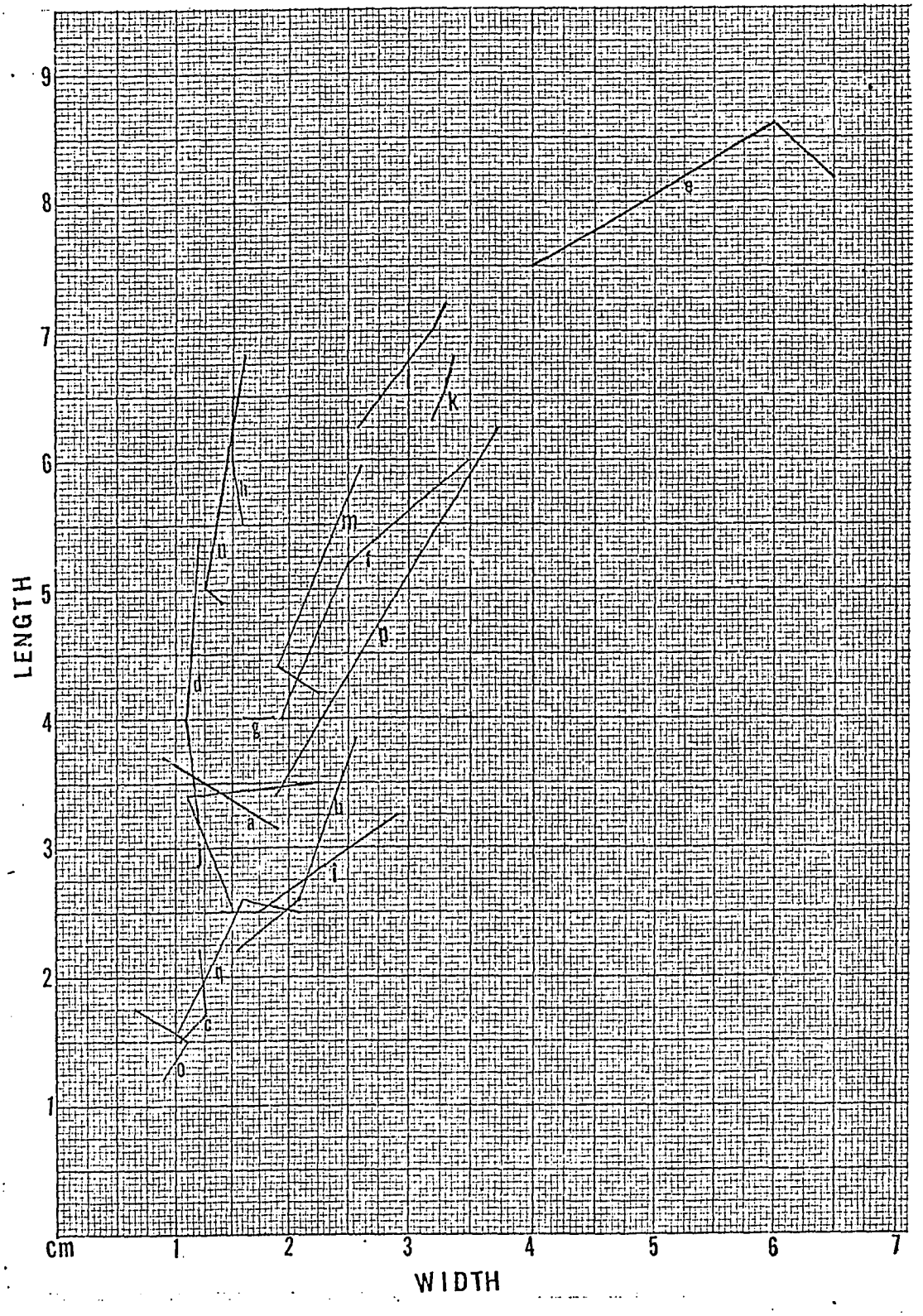

LENGTH
WIDTH
cm
1
2
3
4
5
6
7
8
9
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o

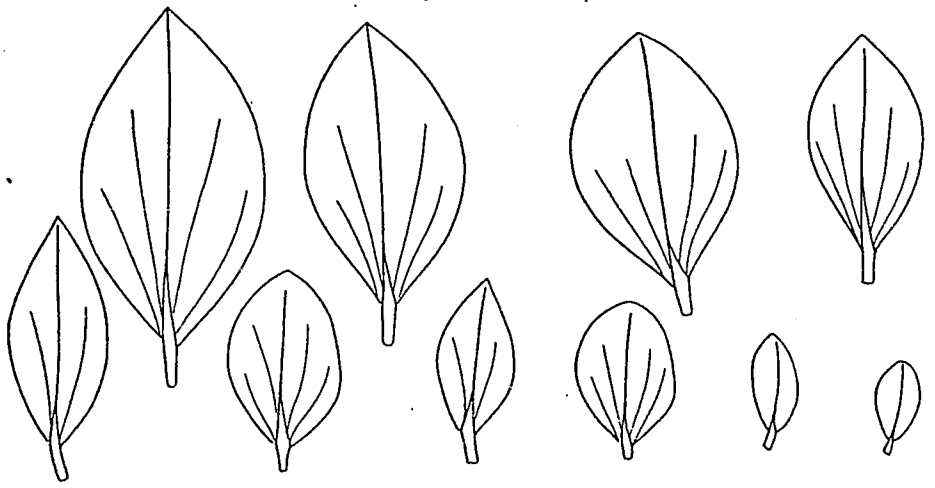
FIGURE 4.

Variation of leaf size and form in:

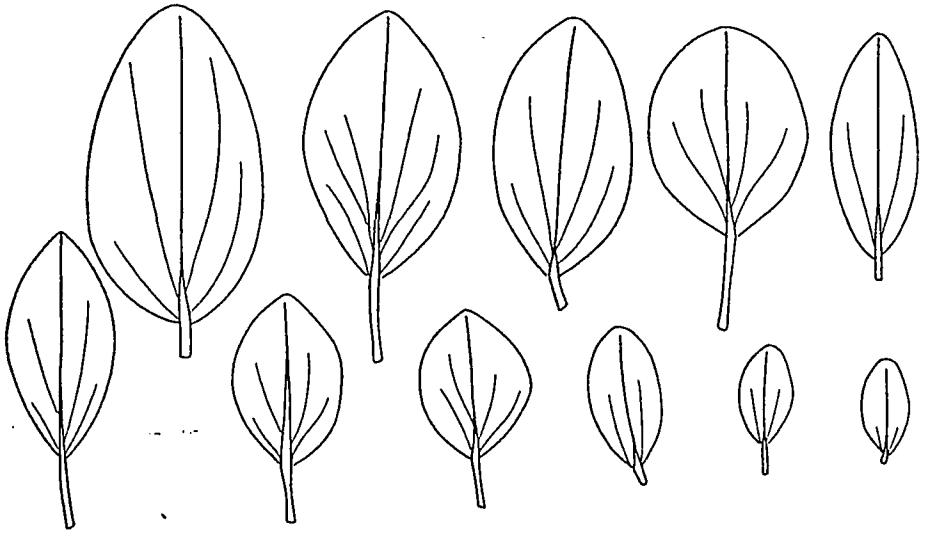
P. leptostachya

P. latifolia

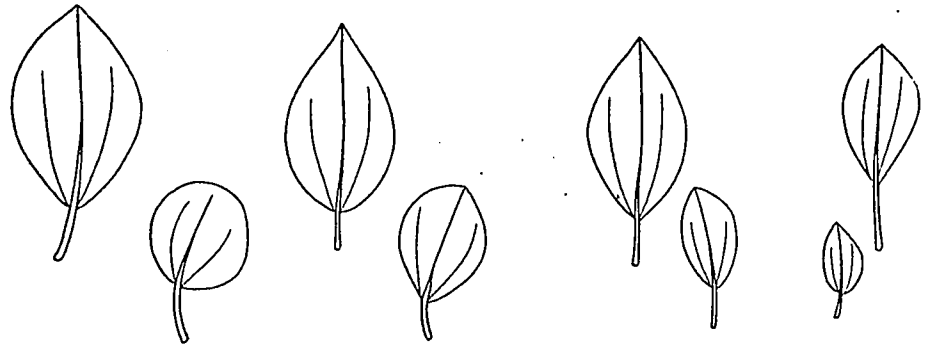
P. cookiana



P. leptostachya



P. latifolia



P. cookiana

10 cm.

FIGURE 5A.

Trichomes morphology of the Hawaiian species of *Peperomia*.

a. *P. ellipticibacca*

b. *P. lilifolia*

c. *P. hirtipetiola*

d. *P. eekana*

e. *P. sandwicensis*

f. *P. hypoleuca*

g. *P. cookiana*

h. *P. leptostachya*

i. *P. latifolia*

j. *P. waikamoiana*

k. *P. erythroclada*

l. *P. macraeana*

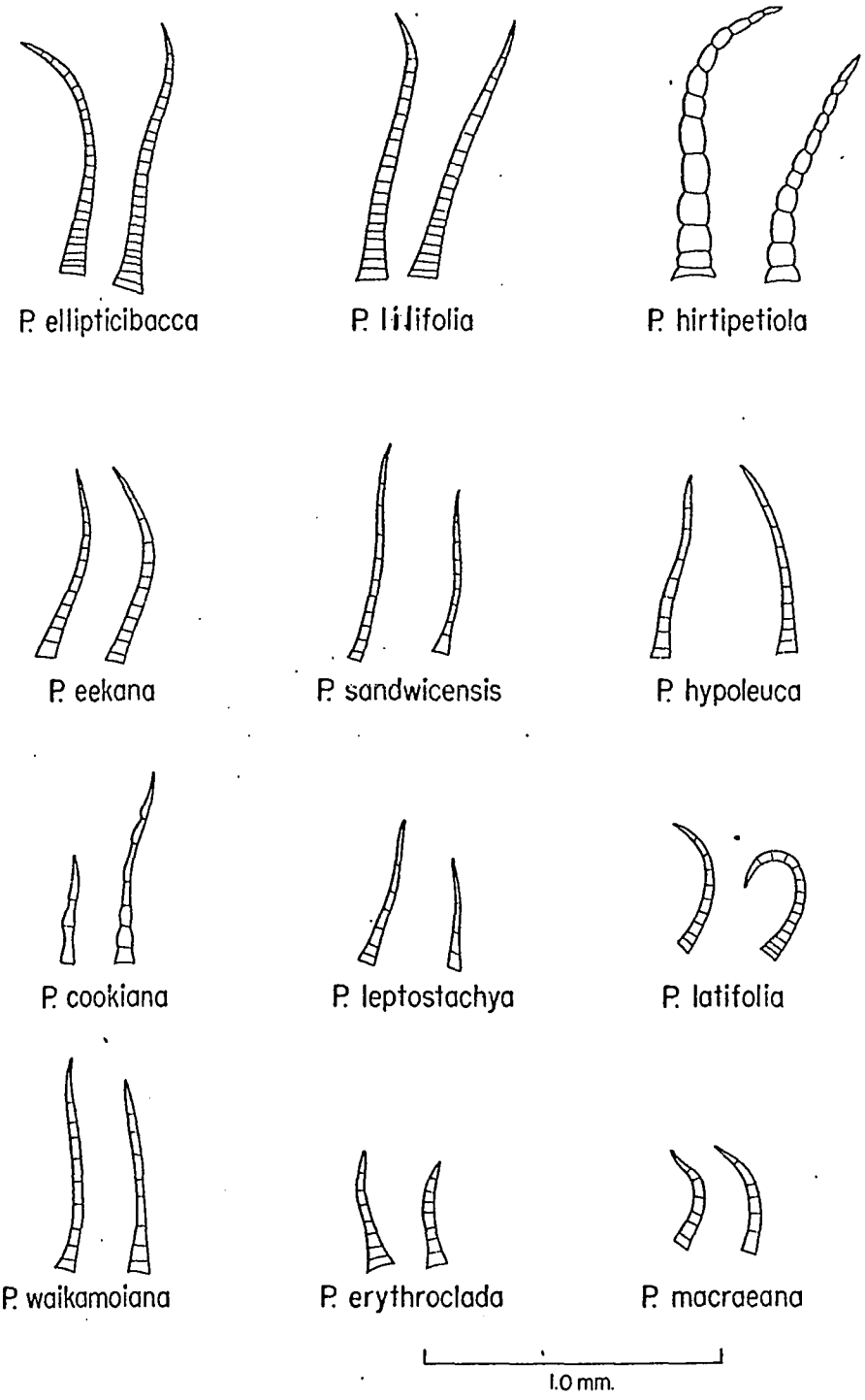

P. ellipticibacca
P. lilifolia
P. hirtipetiola
P. eekana
P. sandwicensis
P. hypoleuca
P. cookiana
P. leptostachya
P. latifolia
P. waikamoiana
P. erythroclada
P. macraeana
1.0 mm.

FIGURE 5B.

Trichomes morphology of the Hawaiian species of Peperomia.

a. P. expallescens

b. P. oahuensis

c. P. kokeana

d. P. remyi

e. P. reflexa

f. P. 241

g. P. 173

h. P. 227

i. P. 176

j. P. latifolia

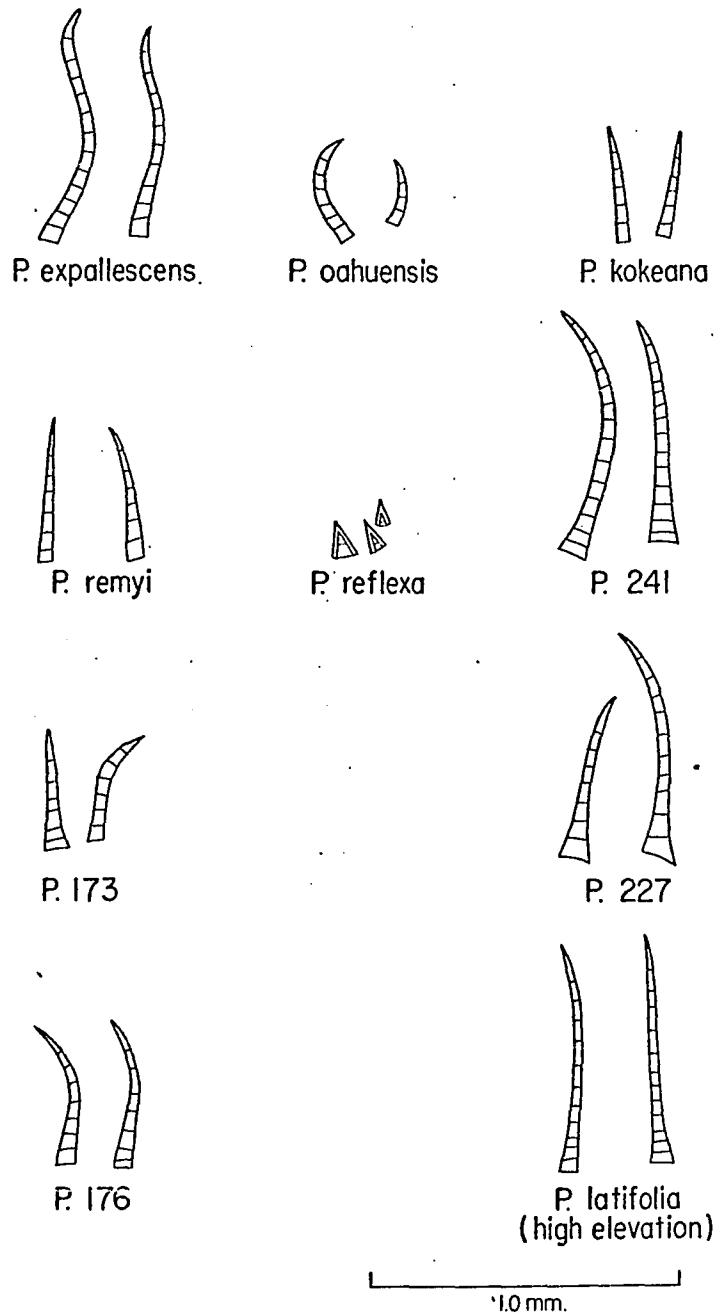

P. expallescens
P. oahuensis
P. kokeana
P. remyi
P. reflexa
P. 241
P. 173
P. 227
P. 176
P. latifolia
(high elevation)
1.0 mm.

FIGURE 6.

Stomata of some Hawaiian Peperomia species showing tetracytic, cruciferous, and rosette types.

a. P. reflexa: tetracytic

b. P. cookiana: anisocytic

c. P. kokeana: anisocytic

d. P. sandwicensis:tetracytic

e. P. eekana: rosette

f. P. lilifolia: rosette

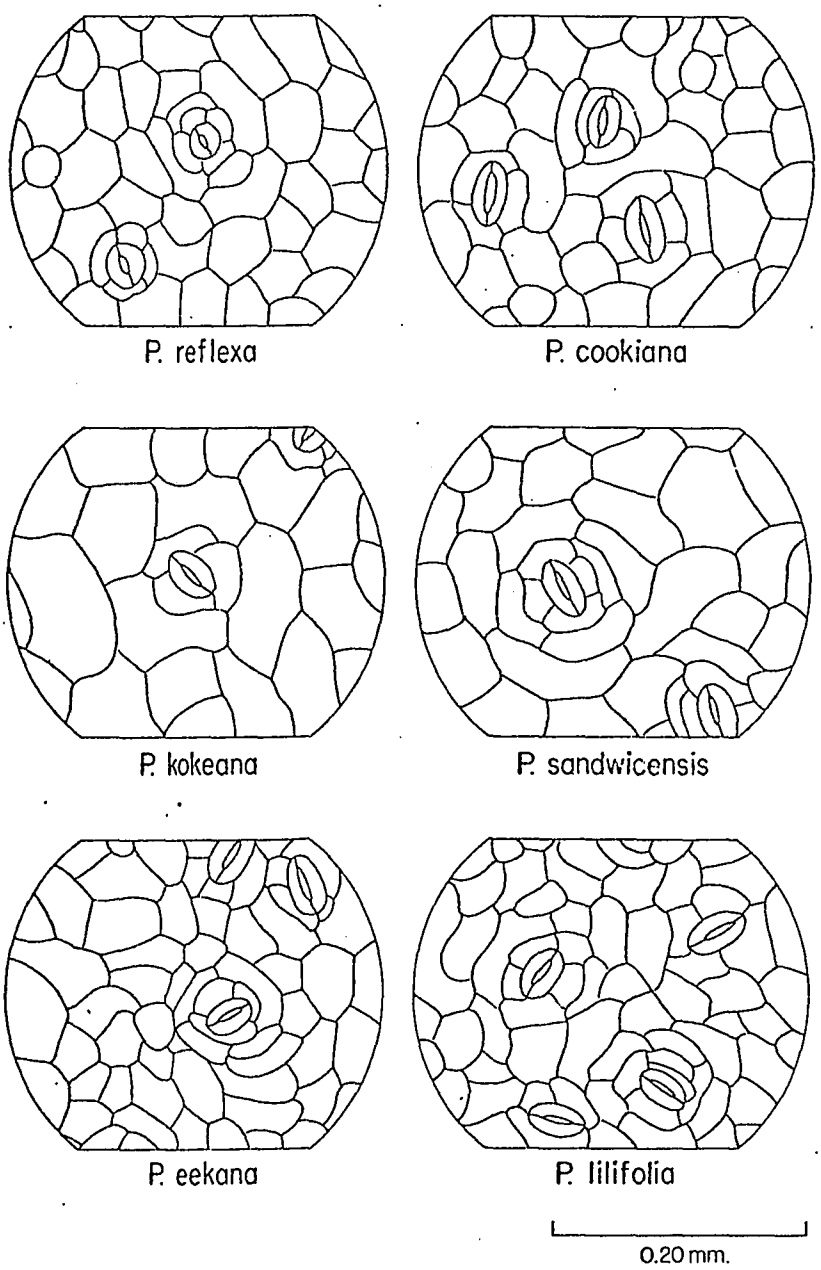
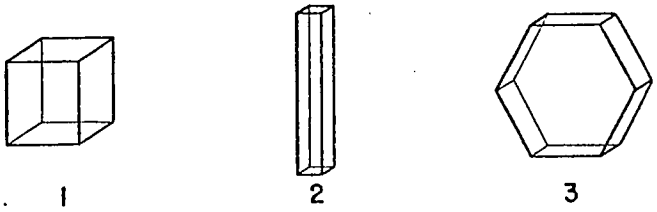

P. reflexa
P. cookiana
P. kokeana
P. sandwicensis
P. eekana
P. lilifolia
0.20 mm.

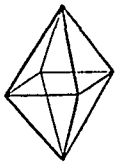
FIGURE 7.

Types of crystals found in the stems of Hawaiian Peperomia species.


1

2

3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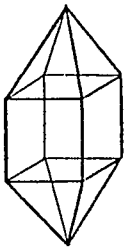
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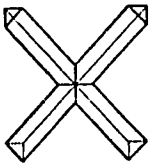
6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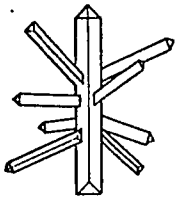
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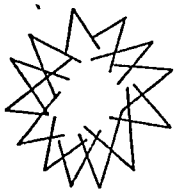
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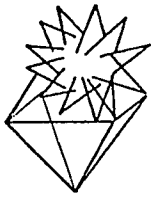
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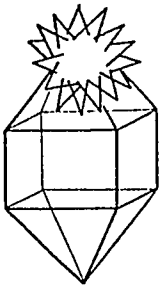

11

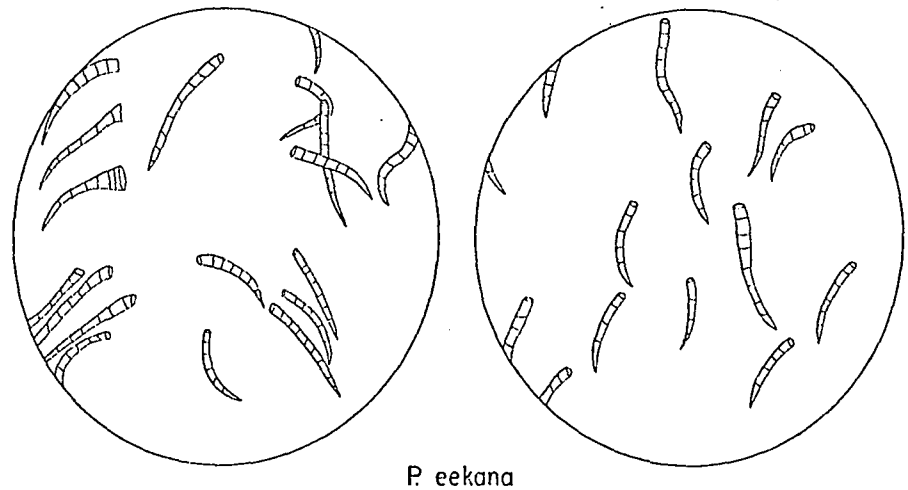
FIGURE 8.

Trichome distribution on the abaxial surface of some Hawaiian Peperomia species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

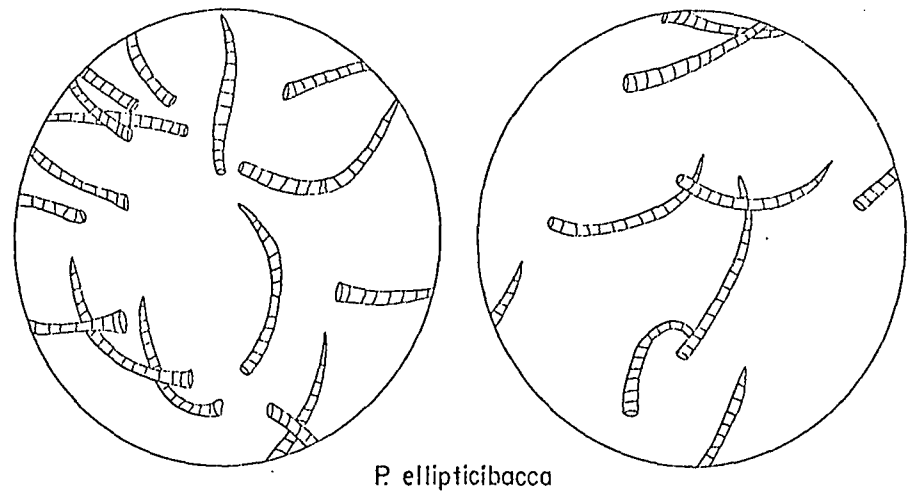
a. P. eekana

b. P. ellipticibacca

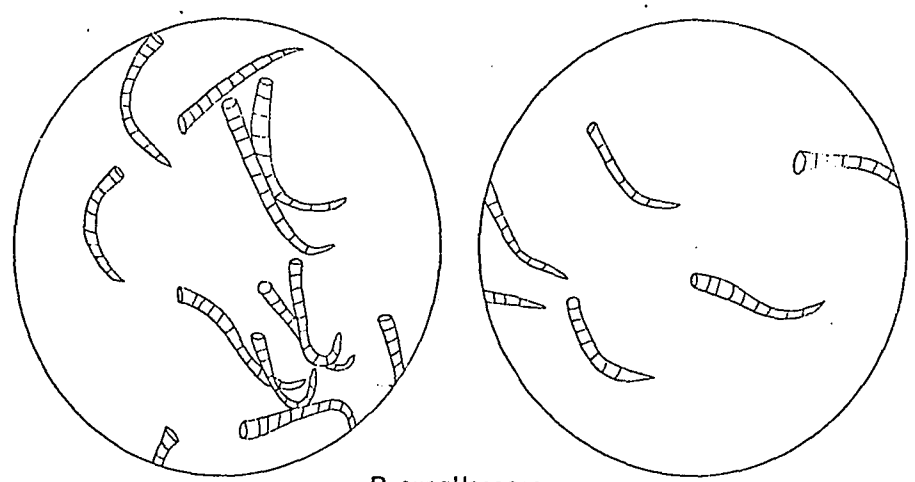
c. P. expallescens



P. eekana



P. ellipticibacca



P. expallescens

1.5 mm.

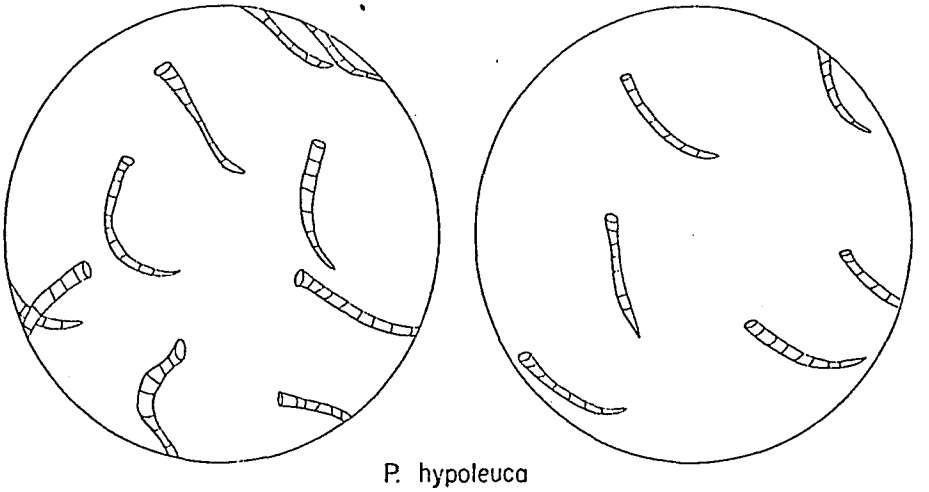
FIGURE 9.

Trichome distribution on the abaxial surface of some Hawaiian *Peperomia* species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

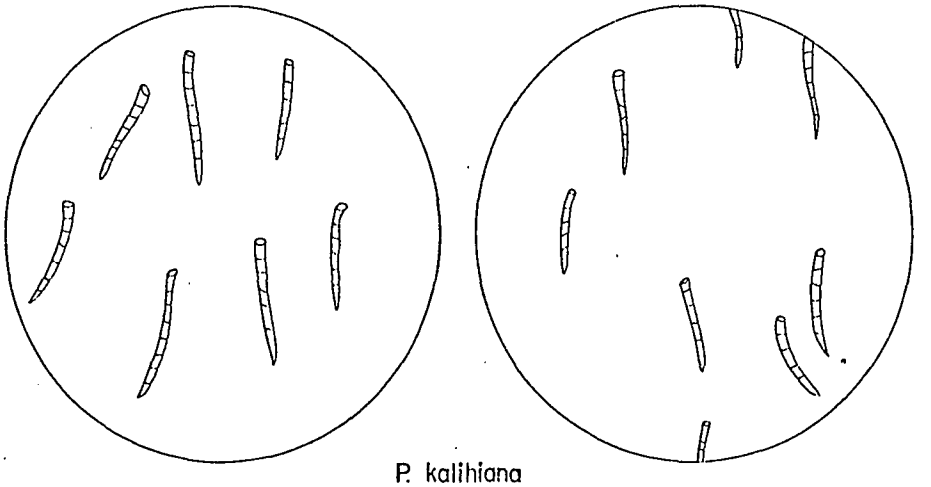
a. *P. hypoleuca*

b. *P. kalihiana*

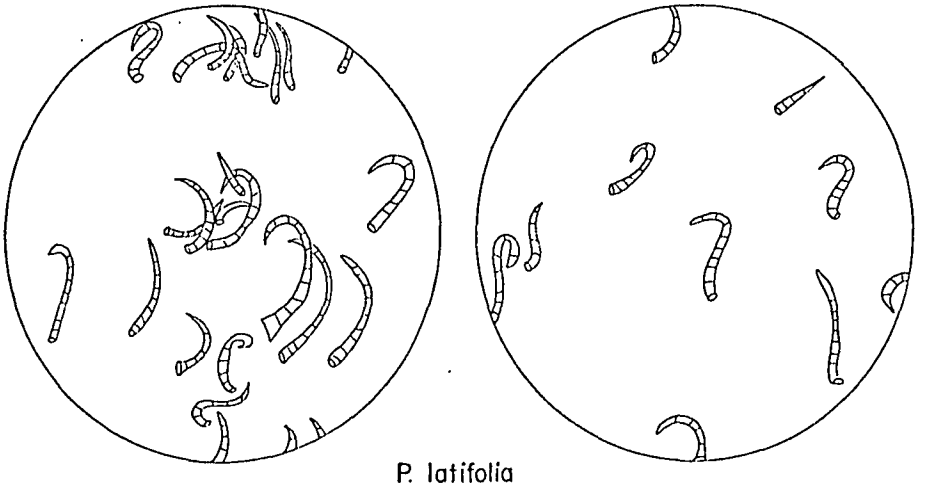
c. *P. latifolia*



P. hypoleuca



P. kalihiana



P. latifolia

1.5 mm.

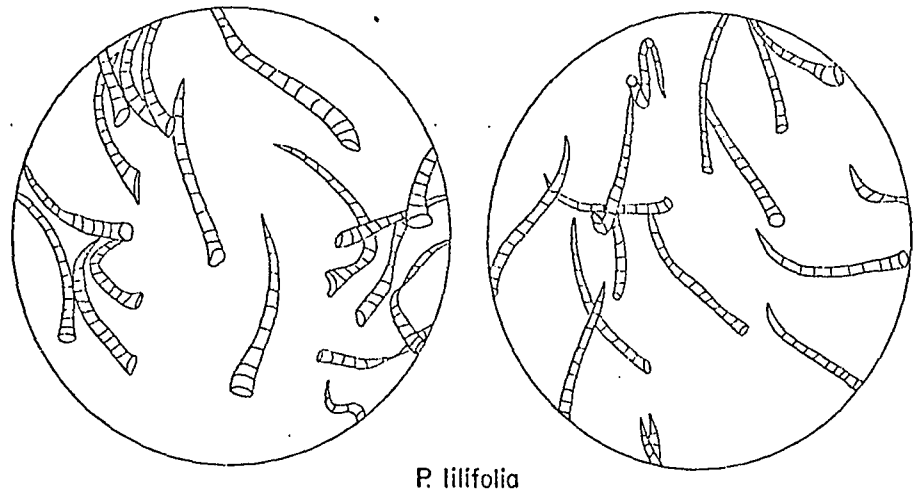
FIGURE 10.

Trichome distribution on the abaxial surface of some Hawaiian Peperomia species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

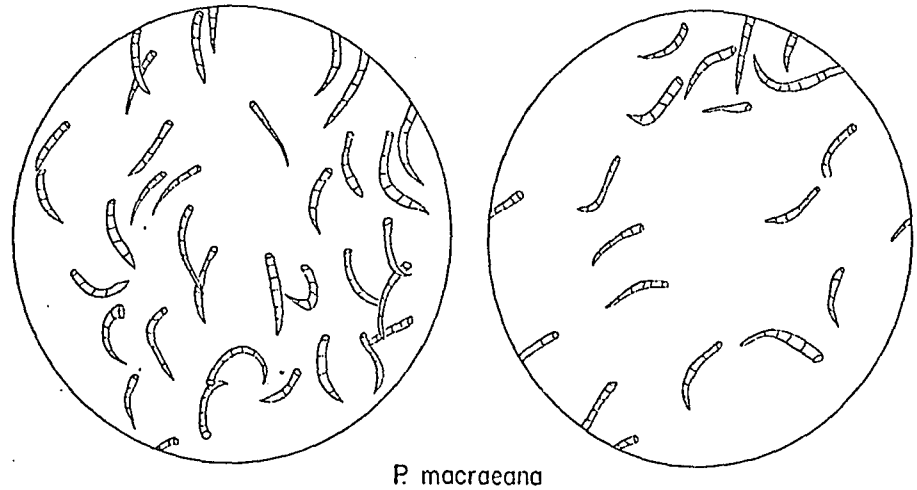
a. P. lilifolia

b. P. macraeana

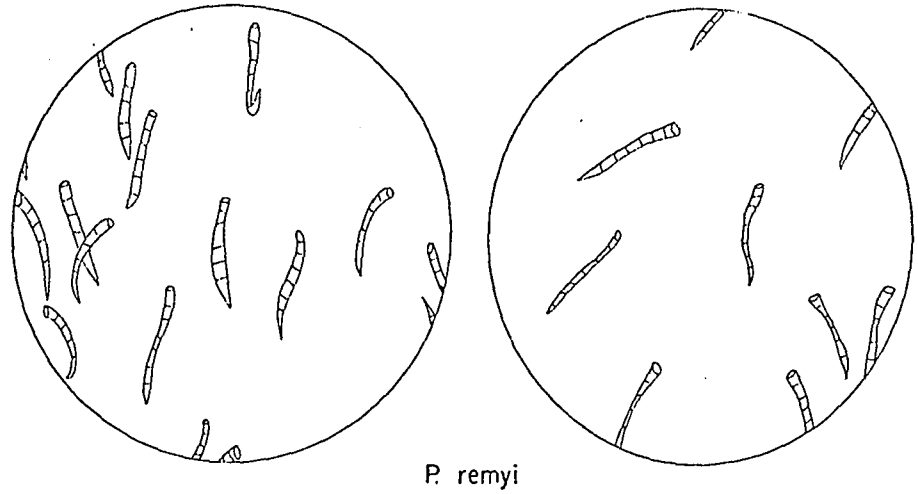
c. P. remyi



P. lilifolia



P. macraeana



P. remyi

1.5mm.

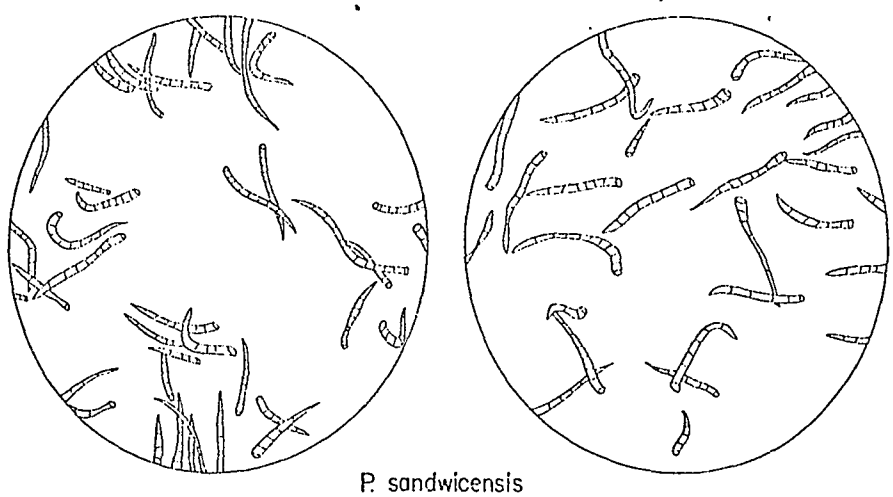
FIGURE 11.

Trichome distribution on the abaxial surface of some Hawaiian Peperomia species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

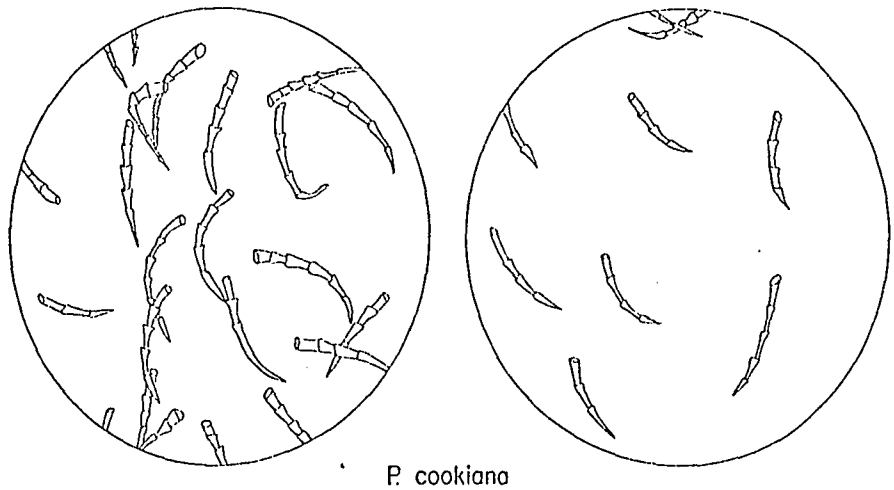
a. P. sandwicensis

b. P. cookiana

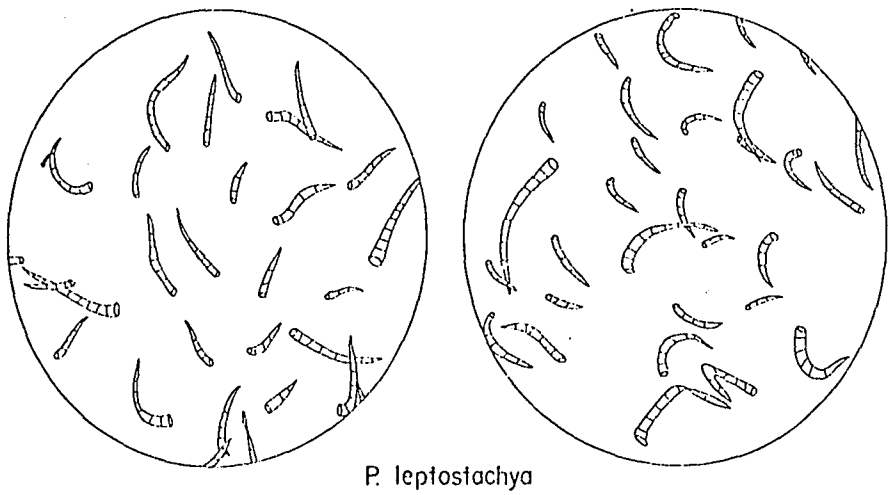
c. P. leptostachya



P. sandwicensis



P. cookiana



P. leptostachya

1.5 mm.

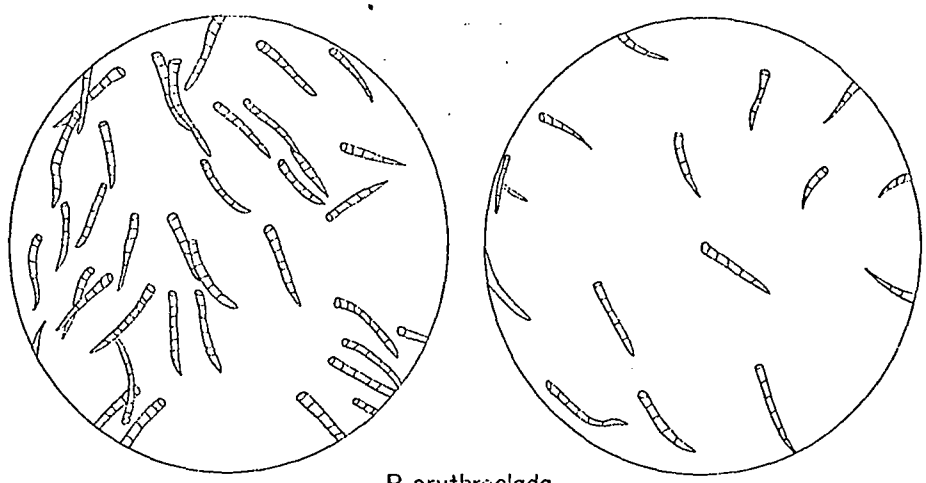
FIGURE 12.

Trichome distribution on the abaxial surface of some Hawaiian *Peperomia* species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

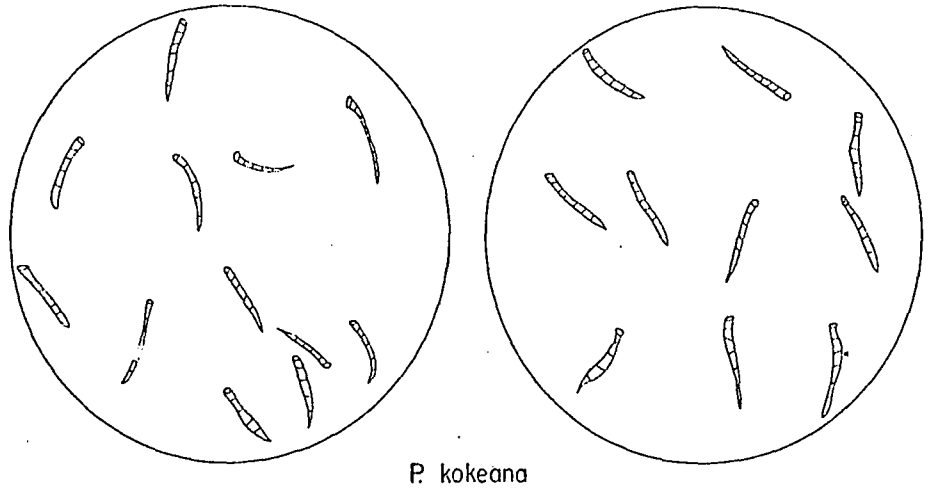
a. *P. erythroclada*

b. *P. kokeana*

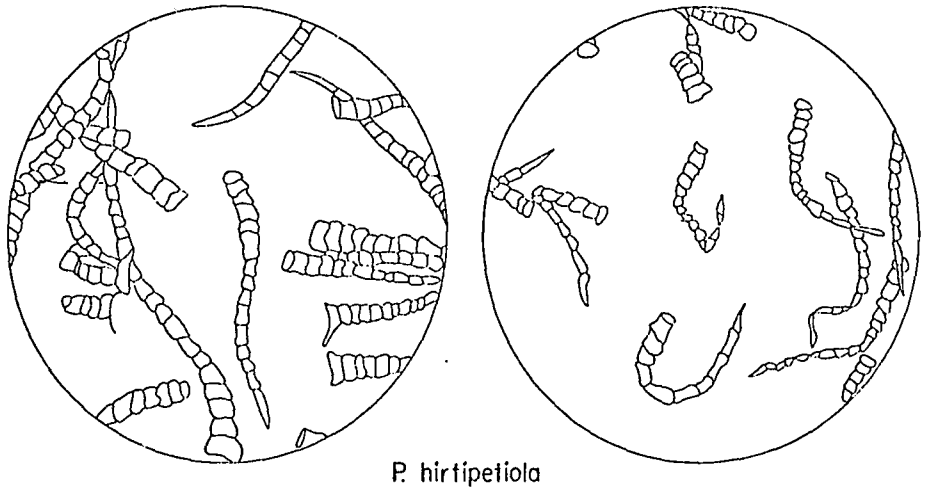
c. *P. hirtipetiola*



P. erythroclada



P. kokeana



P. hirtipetiola

1.5mm.

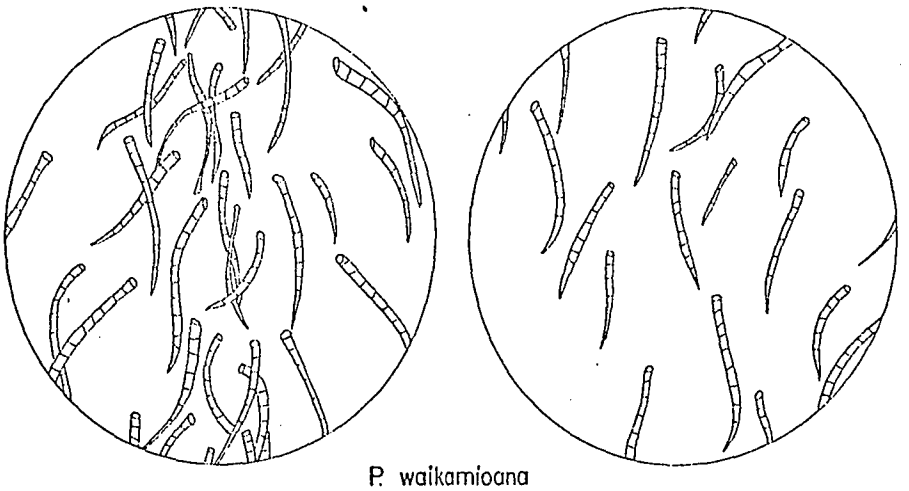
FIGURE 13.

Trichome distribution on the abaxial surface of some Hawaiian *Peperomia* species. The left-hand figure is along the midvein; the right-hand figure is between the midvein and the major lateral vein.

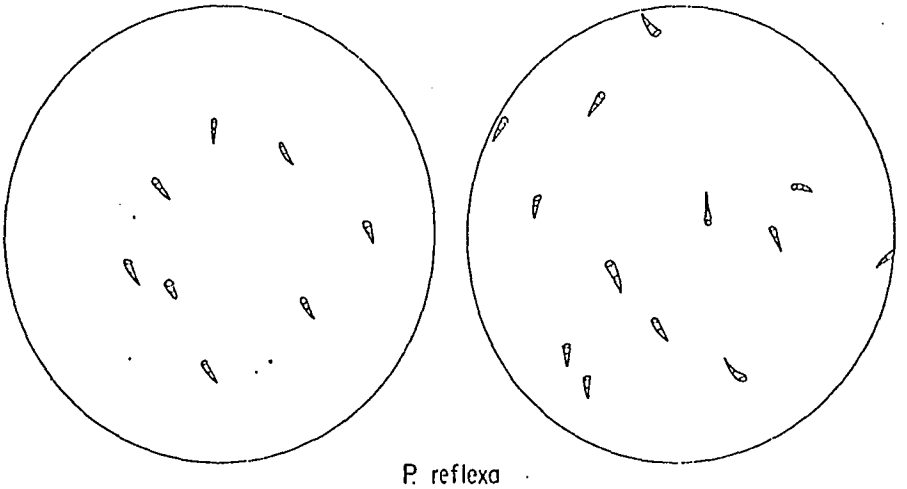
a. *P. waikamoiana*

b. *P. reflexa*

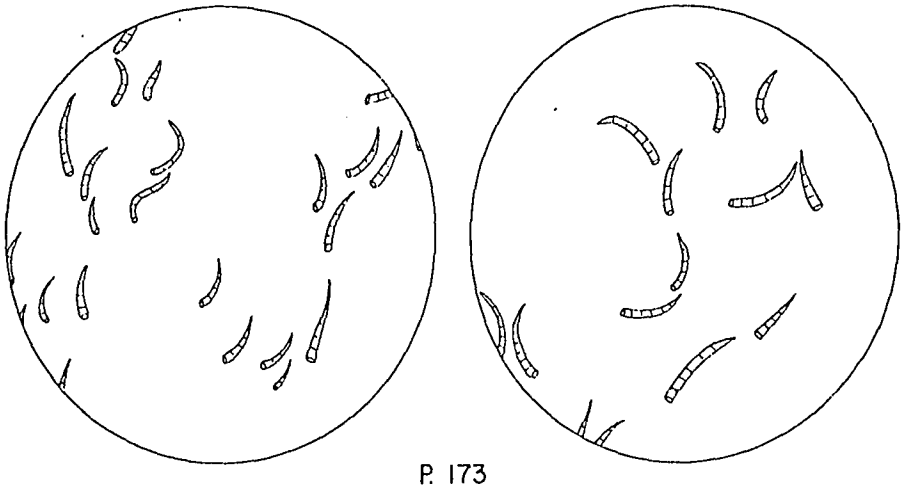
c. *P. 173.*



P. waikamioana



P. reflexa



P. 173

1.5 mm.

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