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# Sexual Incompatibility in Plants

Dan Lewis



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# **Sexual Incompatibility in Plants**

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Edward Arnold

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## Preface

I have written this little book in the hope that some of the pleasure and fascination that the study of incompatibility in plants has given me will come through and will be shared by you. The subject is fascinating because it reveals the great refinement at every level, from molecule to behaviour, of biological evolution which ensures that every environmental niche is exploited to perfection. Even the basic means of attaining this perfection by the recombination of deoxyribonucleic acid is under control at several levels from the molecule itself to the control of inter-breeding. The different breeding systems in plants of which self-incompatibility is the most important and most suited to their special needs, have been developed in great variety. None of these systems are simple; some are extremely complex, and I have not evaded the difficulties but have tried to reduce technical terms to a minimum so that if you have an elementary knowledge of genetics you should be able to understand the more subtle aspects; otherwise my hope would be that you would understand the more obvious and general conclusions and this in turn would rouse your interest in another fascinating subject – genetics.

London, 1978

D. L.

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# 1 Drive for Diversity

'Variety is the spice of life' might have been the first heading and theme of this book, but this implies the trivial and not the essential. Perhaps, 'variety is essential to life' is nearer my meaning, for animals, plants, bacteria and viruses have become what they are and what they will be by the process of evolution through natural selection acting on diverse and varied individuals. For natural selection to be effective, there must be differences between individuals and these differences must be inherited, and therefore they must be genetically determined, or to use a modern concept, they must be encoded in their nucleic acid. Charles Darwin realized the central importance of variation, but explained its preservation by invoking the now discredited hypothesis of the inheritance of acquired characters. Now that we know how inheritance works, we can understand how all organisms from simple viruses to man have developed mechanisms to preserve their variation. Flowering plants are of special interest because they have several different systems of great complexity and subtlety to suit their special needs.

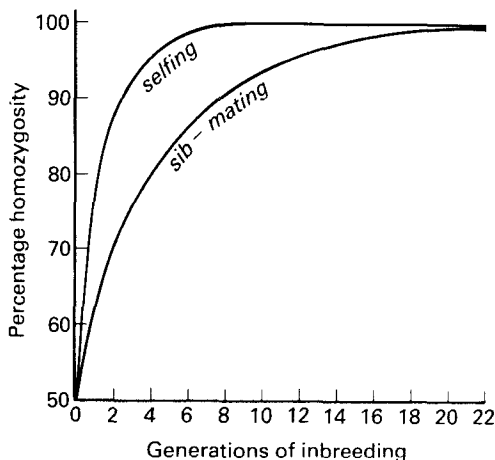
The genetic differences arise in the first place, from sudden random changes in the gene which occur at low frequencies. Single gene mutations may often be effective and useful as immediate sources of variation in bacteria and viruses, where a small colony contains billions of individuals and the time of reproduction is a few minutes; but in higher organisms, the population size is too small, and the life cycle too long for such rare events to provide by themselves a store of variation that can be effective in evolution. A single gene mutation within the genetic background of one individual and one environment is of little evolutionary value; its importance is revealed only when tested in large numbers of individuals and in many environments. Sexual reproduction provides the only effective means of scrambling the genes and their mutations within the imposed limits laid down by the necessity for organisms to have one complete set of genes, if haploid and two such sets, if diploid. Sex does this in two steps; firstly there is a special division of the nucleus, meiosis, preceding the formation of the sex gametes, eggs, sperm, or pollen, which not only halves the number of chromosomes but also rearranges, at random, the two sets of chromosomes which come from the two parents, and also supplies each gamete with a complete set of chromosomes with all the genes they contain. This division also rearranges the genes within a chromosome by the

## 2 DRIVE FOR DIVERSITY

mechanism of crossing-over between parental chromosomes. Secondly, having scrambled the genes into the gametes, the sexual act of mating and fertilization allows the almost random fusion of these gametes to produce virtually unique individuals.

For this elaborate scrambler to work, it must have something to scramble, i.e. there must be normal and mutant genes in the same individual; the individuals must be heterozygous for a proportion of their genes. Heterozygosity can be preserved by the orderly Mendelian segregation of genes, but this requires cross-fertilization between individuals. If self-fertilization occurs, as it can in many plants and some sedentary hermaphrodite animals, such as the oyster, the individuals arising after several generations of selfing are homozygous. Mendel's law of segregation clearly shows how this occurs; if we consider a plant or animal which is heterozygous for one gene, say tall and dwarf in Mendel's pea plant, the offspring after one generation of selfing will be: one tall  $TT$  homozygote, one dwarf  $tt$  homozygote, and two tall  $Tt$  heterozygotes. One generation of selfing has reduced the heterozygotes to one half. If the original plant had been heterozygous for ten genes, then the offspring from selfing, on average, would be heterozygous for only five genes.

Animals, having their sexes in different individuals, cannot self, but they can cross-fertilize between brother and sister. This produces a similar inbreeding effect to selfing but at a slower rate. Cousin mating also produces the same effect but at a still slower rate. Whether it be selfing, brother-sister or cousin mating, the long-term end result is homozygosity, true breeding and little or no variation to scramble.



**Fig. 1-1** The relationship between the number of generations of selfing and sib-mating and the % of homozygous genes.

Figure 1-1 shows the effects over twenty years of two types of inbreeding.

Hermaphrodite plants, for continued evolution, must be able to control self-fertilization by preventing it or reducing it to a low level, but as a temporary measure it may be necessary to resort to self-fertilization for survival under difficult conditions which might prevent cross-pollination. Self-fertilization is not a problem in animals, and brother-sister (sibling) mating is solely controlled by behavioural instincts and taboos. Sib-fertilization in plants has been considered by some authors to be of little importance, but once self-fertilization has been controlled, it can be argued from the limited dispersal of seed and pollen and from the efficient incompatibility systems which do limit sib-fertilization, that it is worthy of consideration. Many ingenious experiments have been carried out with both wind- and insect-pollinated plants to measure the distribution of pollen in distance from the source. They show a remarkable general agreement, in that more than 90% of the pollen is deposited within 1-4 metres of the source, and a small percentage, diminishing with the distance, is deposited over many kilometres. Seeds are similarly restricted.

These two facts are very important factors in the population structure and mating of plants. Most of a plant's close neighbours will be its sibs, most of the pollen that arrives on a flower will be from sibs. This close relatedness of pollen and seeds is affected by other factors which are difficult to assess; the density of plants on the ground and the number of flowers open at any time on the plant are the obvious ones. Wild cherry trees may be further apart than buttercup plants, but the higher number of flowers on the cherry counteracts the bigger distance. Plants, as adults, are static, and their pollen and seeds are carried by wind or animals. The whole economy of sexual reproduction depends upon the intricate adaptation between the plant and the transporting agents, and by these adaptations the plant has not only to control its breeding system but also to reproduce economically without excess wastage of pollen and eggs. The way plants have overcome their limitations is the main subject of this book, but in order to get the full meaning we must consider briefly why the system of separation of the sexes which is so highly successful in animals, has been tested in plants and generally rejected.

About 5% of the flowering plants have male and female sexes in different individuals, the hop, asparagus and cannabis providing examples. These dioecious species occur sporadically throughout the plant kingdom and have evolved from the common hermaphrodite plants. They have not evolved into large and successful groups. Separation of the male and female in separate plants is probably extremely wasteful, for not only does half the population produce no seeds, but the pollen potential must exceed the requirements many



times. This separation of the sexes does, of course, exclude the most extreme form of inbreeding, selfing, but it offers no protection against sib-mating, because pollen from a male plant is equally effective on a female whether it be a sib or non-sib. If, as I believe, the control of sib-fertilization is important, separation of the sexes has a limited value as an outbreeding device in static plants. Animals, with their mobility, behaviour, perception and communication have developed the separation of the sexes to perfection. The vagaries of relying on wind or erratic insects has been replaced by a highly motivated and efficient transfer of sperm by coitus. This provides the necessary economy in reproduction. Animals have instinctive migration behaviours, while man, in addition, has conscious traditions and taboos to control fertilization; plants without mobility, motivation, or consciousness have developed a genetic system of equal or greater efficiency.

## 2 The Promiscuous World of Pollination

Flowering plants have three means of transferring male pollen to female stigma, wind, water and animals. Wind and water were here before the flowering plants evolved; several groups of insects preceded the flowering plants by many millions of years, and these insects, beetles, thrips, sawflies, are still active but inefficient pollinators of flowers. The insects which are specialized for flower feeding and pollination are the *Hymenoptera* (bees and wasps) and the *Lepidoptera* (butterflies and moths) and these appeared at the same time as the flowering plants. We should remember that the plant has two seemingly opposite requirements from pollination: (i) to restrict pollen to the individuals of one species and not waste it on other species, and (ii) to discourage pollen from reaching the stigma of too nearly related individuals of its own species.

If pollination is by wind or promiscuous insects, little can be done by the plant to direct the pollen to the right stigma. But the fact that most temperate trees, as well as all conifers and grasses together with several other groups of plants are wind-pollinated and are still flourishing, shows that the problem has been solved well enough at best to compete with plants that are more efficiently pollinated by bees. It has been achieved by the production of large amounts of light pollen in exposed and wind-swept anthers. The female stigma is large and feathery, offering large trapping surfaces, and the ovary contains one or very few seeds, so that if only two to three pollen grains of the right type land on the stigma, there will be full fertility. Contrast this with a poppy flower with >1000 seeds which therefore requires a few thousand pollen grains, and this can only be obtained by species-constant pollination. Many species of bees, butterflies and moths are extremely species-constant in their visits: this behaviour has been found by direct observation of marked bees, by examination of the pollen loads of bees, and also from the contents of combs in the hive. Parallel with the evolution of constant insects, the plant has reinforced this by making their flowers distinct in shape, pattern, scent, and, to a lesser extent, in colour. Flowers have also evolved intricate structures which present difficulties in the extraction of pollen or nectar, so that a bee becomes a specialist and more set in its habits. The snapdragon requires a bee having enough strength to open the mouth of the corolla tube. The nectar in some clover species can only be tapped by a species of bee with a long proboscis. Species which have

these mechanically closed flowers are invariably pollinated by constant insects—bees, butterflies or moths. The more open type of flower as found in *Ranunculus* are more often pollinated by promiscuous insects. At the same time, flowering plants have evolved both mechanical, temporal and biochemical devices to reduce self-pollination. Many hermaphrodite plants have their sexes in different flowers on the same plant; some plants mature their pollen and eggs at different times. The maize plant is a good example of the effectiveness of temporal and spatial separation of the sexes. The male tassel at the tip of the plant matures its pollen at least a day before the female cobs, which are in the axil of a lower leaf, are receptive. The maize plant only produces one tassel and one or two cobs. Furthermore, the pollen, like much wind-borne pollen is short lived. The combination of all these factors make it a very efficient restriction to self-pollination, but of course, only in plants which have a small number of flowers. It is not the intention of this book to give a full account of these methods. But even if these mechanical devices can in some plants reduce self-pollination, they have no effect on sib-pollination, a type of inbreeding which, because of the extremely narrow distribution of pollen and seeds as we have seen earlier, is of considerable importance. Only genetical-biochemical methods of self-incompatibility can effectively control this, not by controlling pollination, but by controlling fertilization.

## 3 The Drive Against Inbreeding

The majority of flowering plants, being hermaphrodite, produce fertile male spores (pollen) and fertile female gametes (eggs) but many species are unable to reproduce sexually by self-pollination; they are *self-incompatible*. A typical example of the scope and efficiency of one type of self-incompatibility can be found in a field of clover: every plant is self-incompatible but to find a pair of plants which were cross-incompatible would require testing on average more than 22 000 pairs. Not all self-incompatible plants have such an efficient system as the one in clover. There are five main systems known and probably there exist others which have not yet been discovered. They all work either by a specific inhibition of pollen penetration of the stigma, or of pollen tube growth in the style, both of which prevent the male nucleus fertilizing the egg; or more rarely, as in cacao, the egg is fertilized, but early abortion occurs after selfing. There are several logical ways of classifying the systems, but for convenience we will use one based upon morphology; these are *Heteromorphic* in which differences in flower morphology characterize the inter-compatible types and *Homomorphic*, in which there are no such differences.

### 3.1 Heteromorphic incompatibility

#### 3.1.1. *Primrose*

The common European primrose, *Primula vulgaris*, is a good example to illustrate the main features of the heteromorphic system. If you examine the flowers of populations of primroses, except some certain rare populations in Somerset, England, which will be described later, you will find that about half of the plants are long styled, also called pin-eyed flowers, with the rounded stigma at the mouth of the corolla tube and the anthers attached to the tube at about the mid-point; the other half of the plants are short-styled, or thrum-eyed, in which the anthers are at the mouth of the tube and the stigma is at the mid-point. Figure 3-1 shows these two types.

There are other morphological differences which require a microscope to reveal. The pollen grains in the short-styled plant are larger than those in the long-styled plant, they have about twice the volume and, as we shall see later, they have twice the distance to grow to achieve fertilization. The surface sculpturing of the pollen grains is also

distinct. The surface of the stigma of the long-styled plant has larger cells than those of the short-styled. These morphological differences help, but not very effectively, to distribute the pollen from one type to

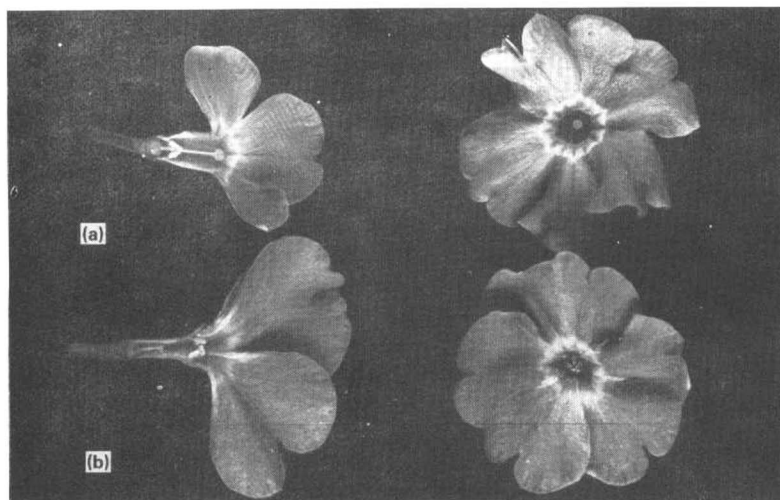
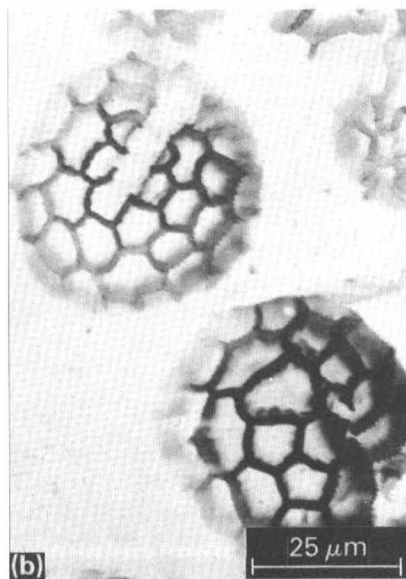
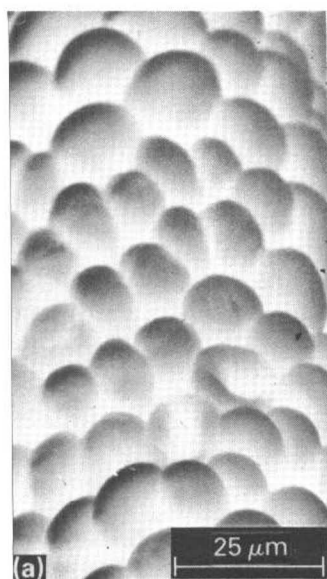
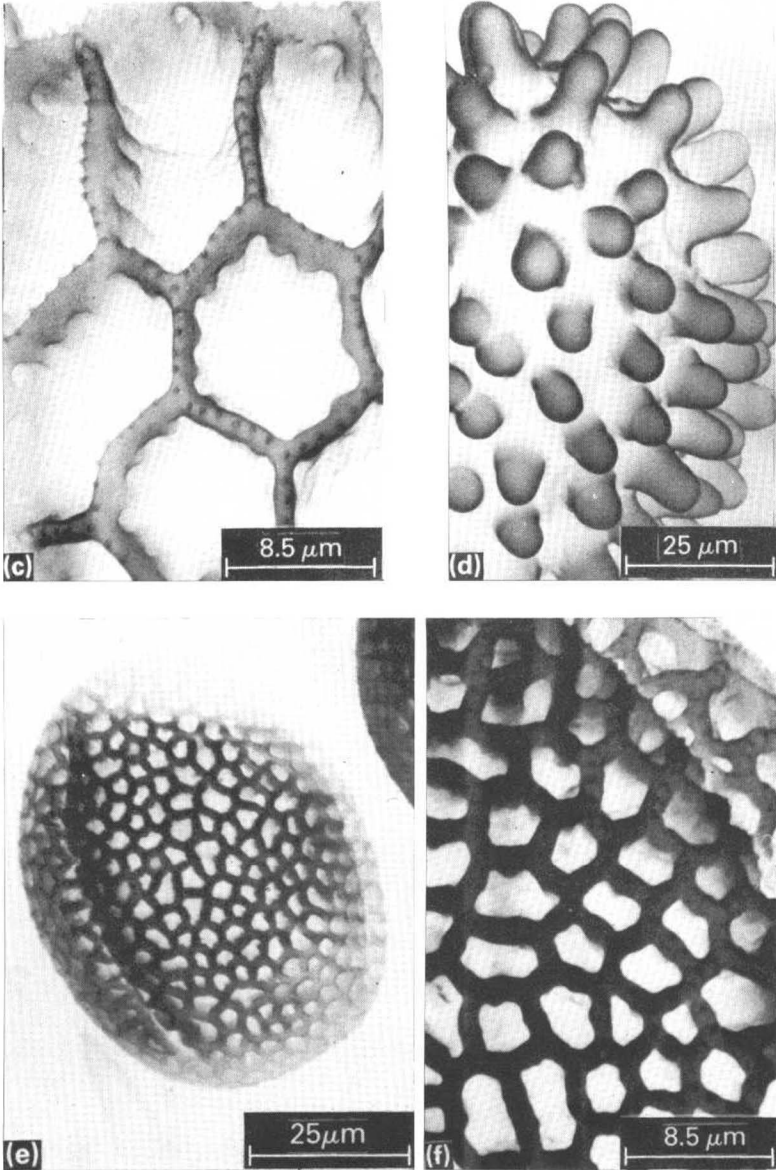


Fig. 3-1 (a) Long-styled (pin-eyed) and (b) short-styled (thrum-eyed) flowers of *Primula obconica*.

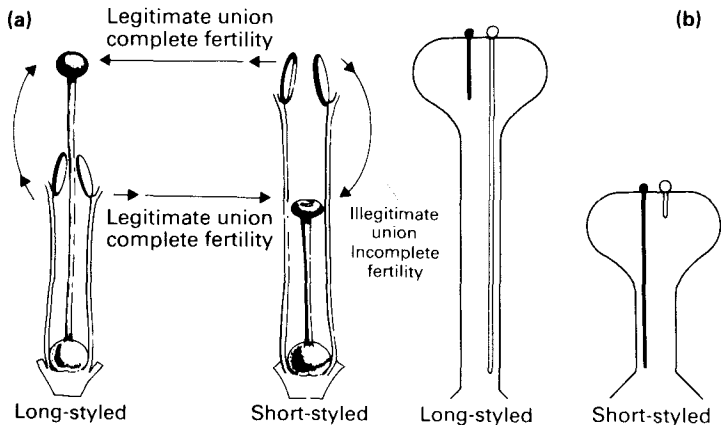




**Fig. 3-2** Scanning electron micrographs of stigmas and pollen in *Limonium meyeri*; by permission of D. R. Dulberger and the Royal Society. (a) Stigma of long style. (b) and (c) Pollen of short-styled plant. (d) Stigma of short style. (e) and (f) Pollen of long-styled plant.

the other. The corresponding height of anthers and stigmas between the two types and the restricted diameter of the corolla tube helps to effect cross-pollination. The size of the stigmatic cells and the pollen sculpturing may help to assist the adhesion of the pollen on the stigma of the other type. These pollen and stigma surfaces are well developed in the heteromorphic species of sea lavender, *Limonium myerii*.

But these morphological differences give only fringe benefits of doubtful value and are the outward and secondary trappings of a much more important difference which is the basis of incompatibility. If the pollen of either the long- or short-styled plants is placed on its own type of stigma, whether it be from the same plant or another plant of the same type, the pollen tube will penetrate the stigma and will grow to 1 or 2 mm into the style and then stop growing, thus preventing the nucleus reaching the egg and hence producing no seeds. This was called by Darwin 'an illegitimate union'. The legitimate or compatible union is between the two types in which the pollen tubes grow at a steady rate, reach the ovary in 18–24 hours and produce seed (Fig. 3–3). The biochemical nature of the inhibition in



**Fig. 3-3** (a) The two forms of *Primula* indicating the two legitimate (compatible) and two illegitimate (incompatible) pollinations (from DARWIN, 1877); (b) the pollen tube growth from the four types of pollination, note the difference in pollen size. ●, pollen of long-styled plant; ○, pollen of short-styled plant.

the incompatible union is discussed later, but it should now be realized that it is a process of self-recognition which results in rejection of self. The consequences in terms of the breeding system and outbreeding are identical to the effect of separation of the sexes in different individuals as in animals and a few plants. The long- and

short-styled types in *Primula* and other distylic species and the two sexes in dioecious species are both in equal proportion in the population. This equality is ensured by the genetic control of sex which is by the two X-chromosomes in the female and an X- and Y-chromosome in the male. In distylic species, the genetic control is by a cluster of genes designated *S* and *s*; the long-styled plant is homozygous *ss*, and the short-styled plant is heterozygous *Ss*. When they are crossed together in the compatible combination they produce long- and short-styled plants in equal numbers:

$$\begin{array}{rcc}
 \text{Parents} & \text{Long} \times & \text{Short} \\
 & ss & Ss \\
 & & \downarrow \\
 \text{Progeny} & 1 \text{ Long} & : 1 \text{ Short} \\
 & ss & Ss
 \end{array}$$

Both distyly and separation of the sexes prevent self-fertilization and fertilization between individuals of the same groups or sex, but neither give any restraint or control on sib-fertilization. This is because the proportion of the two groups is the same in a family and the population as a whole. The major difference between separation of the sexes and distyly is that both types in distyly produce offspring and hence there is a considerable crude economy in reproduction.

Heteromorphic incompatibility is found in approximately 17 families and about 100 genera (see Appendix). The full complex of morphological differences found in the primrose is found in several other genera, including the sea lavender, the pollen and stigmatic surfaces of which are illustrated in Fig. 3 2. Some species, for example, *Linum grandiflorum*, have styles of different lengths, but the anthers are at the same height in the two types and the pollen is of the same size, although the two pollen types differ in their osmotic pressure, which is another possible way to compensate for the different style lengths through which the pollen tubes have to grow. The one common feature of heteromorphic species is the incompatibility.

A comparative study of species and genera in the sea lavender family, the *Plumbaginaceae*, shows that heteromorphic incompatibility has evolved in a sequence from monomorphic species which are self-incompatible through species with dimorphic pollen, dimorphic pollen and stigmatic cells, to the full heterostyly. This indicates that incompatibility predates heteromorphism.

An important feature of self-incompatibility systems is that they can change to self-compatibility without complete disruption of sexual reproduction, and if for some change in the environment self-compatibility becomes an advantage, or even a necessity, then such a change would be selected. For example, if the particular insect pollinator was not active, survival would depend upon the presence of



the rare self-compatible plant. Self-compatible plants do exist in heteromorphic species and they are usually homostyles, i.e. they have the style and stigma of one type and the anther and pollen of the other. These are known as cultivated varieties of several species, such as *Primula hortensis*, *P. obconica* and *P. viscosa*; they probably arose as rare types which were unconsciously selected by growers for their self-compatibility. There are several populations of the primrose *P. vulgaris* which contain 50–60% of self-compatible long homostyles found in Somerset, England. There is no obvious reason for these local



**Fig. 3-4** Long homostyle flower of *Pemphis acidula* from Malagasy. (a) General surface view; note that the stigmas and anthers are both at the top of the tube. (b) Half-section through flower, the anthers have shrunk in the preparation and in the fresh flower are on the same level as the stigma.

populations of homostyle primroses because they occur sporadically and within an area which contains the normal long- and short-styled populations. Perhaps a careful study of the local ecosystems might reveal a correlation.

In *Pemphis acidula* (Fig. 3-4), a plant which only grows in the tropics on coral at sea level and often on isolated islands, most of the populations are typically distylic, but in southern Malagasy the population is composed of 100% long homostyles that are self-compatible. The completely insular environment of this population, and some local situation affecting the normal pollinating insect, or a small founder population following one of