The Ontogeny and Inheritance of Extra Petals in *Potentilla fruticosa* L.

by

Robert Lorne Innes

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ABSTRACT

To investigate the ontogeny of the extra petals, sectioned and intact shoot apices from plants producing single and double flowers were examined using optical and scanning electron microscopy. In both flower types the whorls of floral organs were initiated in a helical pattern similar to that of the foliage leaves, although the time interval between successive primordia was decreased. The petals and stamens were initiated on a pentagonal ridge surrounding the apex. A primordium was initiated at each vertex of this ridge and about five others along each side. Initially there were no apparent differences among these primordia. The primordia at the vertices always developed into petals. In single flowers all other primordia on the ridge developed into stamens. In the double flowers the primordia adjacent to those at the vertices developed petalloid characteristics in the same sequence in which they were initiated. The extra petals are thus found in positions otherwise occupied by stamens, and form at the expense of stamens. Quantitative analysis of petal and stamen numbers supports this conclusion.

A backcross program was employed to test a model for the inheritance of extra petals in Potentilla fruticosa L. pro-

posed by Davidson (1986). Existing F_1 hybrids between four cultivars with varying petal numbers were backcrossed to both parents. Petal number was found to be controlled by two duplicate major genes and at least two modifier genes, one of which was closely linked to the self-incompatibility (S) locus. All of the genes affecting petal number were inherited independently.

The self-incompatibility system operating in P. fruticosa was investigated by studying pollen cytology and by a diallel crossing program. The pollen was found to be binucleate, a condition typical of species with a homomorphic gametophytic self-incompatibility system. The results of the diallel crosses also indicated a single locus with multiple alleles acting gametophytically in the pollen.

An attempt at radiation-induced mutagenesis was unsuccessful, although the LD-50 for rooted cuttings of P. fruticosa exposed to gamma radiation was determined to be 5 krad.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

Potentilla fruticosa L. is a dwarf shrub with a circumpolar distribution in the north temperate zone (Davidson 1986). Several cultivars of the species are used extensively in the landscape due to the low temperature tolerance of this species and its continuous flowering habit throughout the growing season (Rhodes 1954). Although the flowers typically have five petals, those with extra petals (often called double flowers, Reynolds and Tampion 1983) occur in the cultivars P. f. 'Snowflake' (Rhodes 1954), P. f. 'McKay's White' (Fourrier 1985), and P. f. 'Sundance, P. f. 'Snowbird', P. f. 'Yellowbird', and P. f. 'Pink Whisper' developed from the breeding program at the University of Manitoba. Because P. fruticosa is used as an ornamental shrub, the presence of extra petals enhances its aesthetic appeal and therefore the value of a cultivar. The object of this study was to examine the origin of extra petals from a genetic and developmental perspective.

Self-incompatibility (SI) is a breeding barrier in P. fruticosa, and is expected to interfere with seed set in a

backcross program (Davidson 1986). The nature of the SI system operating in this species was investigated using pollen cytology and intercrossing F_1 hybrids between two self-incompatible cultivars.

Inducing mutations through the irradiation of plant propagules is a method of introducing new genetic variabiltity into a population (Van Harten 1982). Robertson (1984) and Davidson (1986) found a genetic limit to petal number in P. fruticosa, and rooted cuttings were therefore irradiated in an attempt to induce a mutation resulting in an increase in petal number.

Chapter II

LITERATURE REVIEW

Double flowers - those containing extra petals - have long been of interest to the horticulturist due to their increased prominence (Reynolds and Tampion 1983). Plants producing such flowers exist in many genera of horticultural importance. Extra petals have been observed in several herbaceous species of Potentilla such as P. tomentilla, P. reptans, and P. hybrida (Reynolds and Tampion 1983, Davidson 1986), as well as in several cultivars of P. fruticosa. (Davidson 1986).

2.1 FLORAL MORPHOGENESIS

The vegetative shoot apex produces one kind of primordium in a regular phyllotactic pattern (Reynolds and Tampion 1983). Conversion of the apex to the reproductive state in some taxa is presaged by a change in leaf shape and/or leaflet number such that leaflet-bract-sepal gradation may be continuous. Cytologically the converting apex may be characterized by an increase in nucleolar volume associated with increased RNA synthesis (Nougarede et. al. 1965, Nougarede (1967). These larger nucleoli may be surrounded by a halo (Sawhney and Greyson 1972).

The reproductive apex sequentially produces several different kinds of primordia, and the time interval between the inception of successive primordia is decreased so that they appear to be initiated in whorls (Reynolds and Tampion 1983). The rates of development of the different whorls of floral organs are not constant. The rapid development of the calyx is consistant with its protective function. Petal primordia generally suspend development at an early stage of differentiation and do not resume development until stamens and carpels are at an advanced stage. Stamens develop quickly, with anther differentiation and pollen production occuring very rapidly, although filament elongation is delayed. Pistil development is also rapid (Reynolds and Tampion 1983).

Floral organs are generally initiated in an acropetal sequence (Reynolds and Tampion 1983) although exceptions do exist. Cheung and Sattler (1967) reported that in Lythrum the inception of the inner calyx preceded that of the outer calyx, and that stamen initiation preceded petal initiation. It has been theorized that such centrifugally initiated organs may be initiated on, or axillary to, existing primordia, rather than on the floral receptacle (Cheung and Sattler 1967).

In several species the proximal portion of the calyx appears to have a receptacular nature with petals and stamens initiated on the adaxial margin of the calyx tube (Jackson 1934, Cheung and Sattler 1967).

The sequence of differentiation of floral parts is generally acropetal, although in many cases such as Aquilegia vulgaris petal differentiation may follow stamen differentiation. In this species the stamens also differentiate in a basipetal direction (Reynolds and Tampion 1983).

Fragaria vesca is a species closely related to Potentilla. Sattler (1973) studied organogenesis of the flower of this species and noted that the inception of the inner calyx preceded that of the outer calyx. The other floral organs were initiated in an acropetal sequence. Three stamen primordia were initiated on each of five elongate androecial primordia which were pentagonally arranged about the floral apex and separated by petal primordia. One additional stamen was initiated adaxial to and opposite each petal primordium.

2.1.1 Origin of Extra Petals

The development of double flowers has been studied in several taxa including Petunia (Natarella and Sink 1971), Dianthus (Emino and Rasmussen 1971, Garrod and Harris 1974), Pelargonium (Wetzstein and Armitage 1983), and Rosa (Moe 1971, Horridge and Cockshull 1974, Lindenbaum et. al. 1975). Reynolds and Tampion (1983) have classified double flowers according to the origin of the supernumerary petals and cite two instances of extra petals in herbaceous species of Potentilla as being due to petallody of stamens and/or carpels (Class IIIb or c).

Reynolds and Tampion (1983) classified double flowers into five classes as follows:

I) Polypetalous plants

a) Those flowers in which the area of the petals is increased by fluting, overlapping, dissection, or outgrowths of the petals.

b) Extra petals are formed in the normal whorl of the corolla.

c) Extra whorls of petals are formed in the normal corolla area.

d) Extra petals result from secondary growth centers on the receptacle.

II) Sympetalous plants

a) The area of the petals is increased as in Class Ia.

b) Extra whorls of petals are formed as in Class Ic.

III) Both polypetalous and sympetalous plants with normal growth in the petal area of the receptacle, but with petallody of some or all of the reproductive organs.

a) Plants with flowers characterized by petalloid outgrowths of the stamens.

b) The stamens are completely replaced by petalloid structures.

c) Petalloid carpels are present.

d) Both the stamens and carpels are replaced by petalloid structures.

IV) The composite flowers, in which pseudo-doubles may result from the replacement of the hermaphroditic tubular florets by pistillate ligulate florets. The degree of such replacement may decline in the later part of the season, allowing some pollen production.

V) Other types of double flowers, such as those resulting from proliferating floral axes (eg. Calendula officinalis prolifera, Reynolds and Tampion 1983).

In sympetalous taxa such as Petunia, doubleness results from proliferation and convolution of the corolla as well as from secondary centers of meristematic activity on the receptacle, filaments, and the adaxial surfaces of the petals (Reynolds and Tampion 1983). This proliferation of petal tissue physically inhibits carpel development resulting in female sterility (Natarella and Sink 1971). The prevention of seed production contributes to the longevity and thus the horticultural value of the flowers.

In all cases cited by Reynolds and Tampion (1983) petalloid stamens were observed situated between the petals and stamens and exhibiting characteristics of both. All grades of structures intermediate between petals and stamens have been observed in many species (Reynolds and Tampion 1983). In most cases the petalloid stamens produced a reduced ammount of viable pollen, although no pollen was noted in Aquilegia (Reynolds and Tampion 1983).

In some taxa such as Rosa an increase in petal number is associated with a decrease in stamen number. For example R. rugosa has five petals and over two hundred stamens, where R. 'Queen Elizabeth' has 105 petals and only 80 stamens (Reynolds and Tampion 1983). Davidson (1986) noted a similar inverse relationship in P. fruticosa. Conversely Lammerts (1945) found that although petalloid stamens do occur in peaches, extra petals were not formed at the expense of stamens, and similar findings were reported by Reynolds and

Tampion (1983) with Aquilegia. A negative correlation between petal number and stamen number does not necessarily imply the conversion of stamens to petals, but could also be due to a prolonged petaliferous stage followed by an attenuated staminiferous stage due to limitations in the nutrient supply. As the developmental strategy may be to maximize seed production this limitation may have a greater effect on stamen than on carpel initiation (Reynolds and Tampion 1983).

Petalloid stamens occur in a helical arrangement consistant with the sequence of initiation of primordia on the floral apex. This may indicate a delay in activating genes for stamen differentiation so that a primordium is initiated under petal forming influences and develops under stamen forming influences or vice versa (Reynolds and Tampion 1983).

Sinnott (1960) states that the fate of a cell is a function of it's position, and Reynolds and Tampion (1983) apply this concept to primordia as well as cells, and state that this effect is mediated by plant hormones. The differentiation of a primordium into a petal, stamen, or a structure intermediate between the two is therefore dependent on the hormonal balance in that primordium at the time of determination.

Sawhney (1983) noted that gibberellic acid (GA) increased the number of all floral organs when applied to flower buds of tomato, and that this effect was greater at higher temperatures. Low temperatures duplicated the effects of GA, indicating that the effect of low temperatures could be due to increased GA production, or that higher temperatures could result in increased production of GA inhibitors. The effects of both GA and temperature were genotype dependent.

Raman and Greyson (1977) reported that flower bud tissue of double flowered genotypes of Nigella damascena contain ten to twenty times the ammount of GA found in single flowered genotypes, and that the types of GA differed between the two types. In seedling tissues, the methanol extractable GA was approximately equally divided between the acidic-ethyl-acetate soluble (AES) and highly water soluble (HWS) fractions. At flowering, the double flowered genotypes contain almost all of the GA in the AES fraction, and the single flowered genotypes contain almost exclusively the HWS fraction. Cultured buds of single flowered genotypes differentiated all floral organs on a medium devoid of growth regulators, but double flowered genotypes required the addition of GA or kinetin to the medium, indicating that doubleness in this species is associated with a change in GA metabolism (Raman and Greyson 1978).

Sawhney and Greyson (1979) found that the differentiation of stamen primordia in the stamenless-2 mutant of tomato

could be altered by the application of exogenous plant hormones at an early stage of floral development. GA caused the mutant stamen primordia to develop into normal stamens, and auxin (IAA) caused these primordia to develop into carpels.

Hormonal modification of floral organs has it's most practical use in the control of sex expression in the cucurbits. Applications of GA can induce staminate flowers (Pike and Peterson 1969) and ethylene can induce pistillate flowers (Robinson et.al. 1970). From the similar effects of hormonal influences on sex expression and the formation of supernumerary floral organs, and from the lack of stamens in the more extreme forms of doubleness (Reiman-Philipp 1983), Reynolds and Tampion (1983) conclude that there must exist some relationship between the structure of the flower and it's breeding behavior from a developmental as well as a structural point of view.

2.2 INHERITANCE OF EXTRA PETALS

The ultimate origin of the heritable double flowering characteristic in any species is by mutation, either in the wild or in cultivation. As the structure of most double flowers is such that pollination is impeded either because of the reduced number of reproductive parts as in Begonia (Bateson and Sutton 1919) or Petunia (Ewart 1984), or by their obstruction by convoluted or supernumerary petals, the sur-

vival of such genotypes is precarious unless aided by human intervention (Reynolds and Tampion 1983). In some instances such as is the case with some double cherry cultivars, triploidy may result in complete sterility due to meiotic irregularities. This is also true of Potentilla fruticosa cv. Snowflake. As fertilization and the initiation of seed development is usually accompanied by petal senescence and abscission, complete or partial sterility may increase the longevity of the flowers, thus enhancing their horticultural value (Reynolds and Tampion 1983).

There exists a great deal of variety in the manner in which the double flowering character is inherited. In his review of previous work in this area, Beatty (1937) cites 36 species in 29 genera in which the inheritance of extra petals had been studied. Various modes of inheritance are reported involving one or two factor pairs, with doubleness being either dominant or recessive in different species. In many species the inheritance of extra petals is complicated by modifier genes (Beatty 1937, Nugent and Snyder 1967), extra-nuclear factors (Rousi 1968), or chromosomal aberrations (Reynolds and Tampion 1983).

In Matthiola incana petal number is controlled by a single locus with the gene for doubleness being completely recessive (Reynolds and Tampion 1983). An 'ever-sporting' strain of this species is characterized by the lack of a satellite on the chromosome containing the S allele at this

locus, and the lack of this satellite renders the pollen inviable, although such a chromosome can be transmitted maternally. The result is that upon selfing, heterozygous singles of this strain will yield single and double progeny in approximately equal numbers, while heterozygous singles with the normal chromosome will segregate in the expected 3:1 ratio. Genetic ratios are also modified due to the increased storage life and seedling vigor of seeds with the gene for extra petals (Reynolds and Tampion 1983).

Chromosomal differences were also noted by Abo El-Nil and Hildebrandt (1973) who showed that anther culture of three double-flowered tetraploid cultivars of Pelargonium hortorum regenerated single flowered diploids. This effect of gene dosage is consistent with the findings of Nugent and Snyder (1967) that the production of extra petals in this species is controlled by a dominant factor at a single locus, but that the number of extra petals produced is controlled by three recessive modifier genes acting in an additive manner. In the polyploid garden dahlia (Dahlia variabilis) Crane and Lawrence (1938) reported that the degree of doubleness was due to the interaction of at least four genes.

In another horticulturally important species, Petunia hybrida, the double flowering character is governed by one dominant gene which also causes female sterility when homozygous (Ewart 1984). Among woody Rosaceous species, Sampson and Cameron (1965) found that the double character in orna-

mental crabapples was also controlled by one dominant gene, but the extent of doubleness was determined by unspecified modifier genes. Lammerts (1945) found one gene and one modifier, both recessive, to control petal number in peaches. In Fragaria vesca, a herbaceous species closely related to Potentilla, extra petals are controlled by a recessive factor at a single locus (Richardson 1917, cited in Beatty 1937).

Potentilla fruticosa is a highly plastic species for many characters of plant form (Davidson 1986), and while the basic reproductive characters are sufficiently stable with respect to size and shape to indicate the taxonomic relationships of the species (Bradshaw 1965), the double flowering trait is environmentally unstable (Robertson 1984). This is not unusual. In many species petal number can vary greatly within one plant. Clarkia elegans and Mecanopsis spp. are examples cited by Reynolds and Tampion (1983) of taxa in which developmental and environmental factors are implicated. The capacity of plants to modify their phenotype under environmental influence is genetically determined in that the general pattern of development may limit the degree to which a trait may be modified (Bradshaw 1965), so that the degree of variation will differ among species. In species where plasticity is a major factor in determining the phenotype, environmental factors may limit the expression of a character.

Hicks (1983) induced petal duplication on cultured tobacco flower buds by surgically bisecting young petal primordia. This technique was only effective in the early stages of flower development when the petal primordia were smaller than the stamen primordia. The effect was localised in the damaged primordium and was attributed to a repair response.

Temperature has a profound effect on the expression of extra petals in many taxa. Low temperatures have been found to result in extra petals from secondary growth centers on the receptacle in Dianthus (Garrod and Harris 1974) and Rosa (Moe 1971, Lindenbaum et. al. 1975). These conditions produce "bullhead" flowers in which the petals are misshapen and the calyx is usually split due to the proliferation of the tissue within. Baer and Kho (1971) found temperatures over 18 C to cause reversion from double to single flowers in Freesia.

Environmental effects on the expression of extra petals in Potentilla fruticosa were studied by Robertson (1984). High temperatures at the time of flower bud initiation were found to increase petal number, and moisture stress at that time decreased petal number. Both effects were genotype dependent. Petal number was also found to be variable over the season, with the first and last flowers to open having a lower petal number than the mid-season flowers. This effect is not rare (Reynolds and Tampion 1983) and is used in Petunia to allow seed production from female sterile double

flowers (Ewart 1984). The double flowering characteristic may require several growing seasons to stabilize as is the case with the Barnhaven strain of double primroses (Sinclair 1971, cited in Reynolds and Tampion 1983) which are single the first year and double thereafter. Double cultivars of Clematis also produce only single flowers until the plants are well established (North 1979). In roses the double flowering character may require two to three years to stabilize (Reynolds and Tampion 1983).

Petal number can be related to plant vigor, and thus decreased by any factor which causes a reduction in the vigor of the plant such as temperature or moisture stresses or inbreeding depression (Reynolds and Tampion 1983).

Strickberger (1985) states that given a fifty percent environmental influence on phenotype, it is impossible to determine the number of genes operating due to distortion of class distinctions. Environmental and genetic effects may be separated with controlled breeding experiments but these methods require breeding lines homozygous for the genes under consideration.

Davidson (1986) investigated the inheritance of extra petals in Potentilla fruticosa and proposed the following model. The production of extra petals is controlled by two independent genes. Either gene in the homozygous recessive condition will result in the production of up to five extra

petals. Petal number may be further increased by the action of a modifier gene which will result in the production of up to an additional five petals when in the homozygous recessive condition.

2.3 SELF-INCOMPATIBILITY

Self-incompatibility (SI) refers to the inability of plants producing functional gametes to set seed when self pollinated or crossed with certain closely related plants (Frankel and Galun 1977). The phenomenon is thus not restricted to "self" incompatibility but is a general mechanism to enforce outbreeding. SI has only recently been used in plant breeding for the production of F1 hybrids in ornamentals (Reimann-Philipp 1983) and cole crops (Pearson 1983), and for the breeding of ornamentals with a prolonged flowering period due to lack of seed set (Frankel and Galun 1977). Much effort has been expended in attempting to solve the problem of developing inbred lines in species where SI systems are operative (Ferran and Wallace 1977, Frankel and Galun 1977, Ockendon 1978, Roggen et. al. 1972, Roggen and Van Dijk 1976).

Species exhibiting SI mechanisms may be classified into two broad categories. Homomorphic species produce only one flower type and heteromorphic species produce two or more different flower types on different plants (Frankel and Galun 1977). The most extreme example of heteromorphism is

dioecy where any given plant will produce only staminate or pistillate flowers. In some species this phenomenon may be subject to environmenal or hormonal modification as in Cannabis sativa (Heslop-Harrison 1956) or in the Cucurbitaceae (Pearson 1983, Pike and Peterson 1969, Robinson et. al. 1970).

Heteromorphism may be manifested as heterostyly where styles of different lengths are found in different flower types, and pollinations within the same flower type (morph) are incompatible. The distyly of Primula is a pleiotropic effect of the S locus controlling the incompatibility response. "Thrum" flowers with short styles are produced by plants heterozygous (Ss) at this locus, and "pin" flowers with long styles are produced by homozygous recessive plants (ss). The reaction type of the pollen is controlled by the sporophyte which produced it so that intermating these types in either direction produces pin and thrum plants in approximately equal numbers (Frankel and Galun 1977). Ordnuff (1980) also found this situation to be the case in Gelsemium sempervirens.

Homomorphic incompatibility systems may be controlled sporophytically in both the pistil and the pollen, or they may be under gametophytic control in the pollen. In those systems controlled by the sporophyte all manner of dominance relationships are possible. Many species in the Asteraceae and the Brassicaceae exhibit this type of SI system (Frankel

and Galun 1977). In the sporophytically controlled SI system in Brassica, there exist about 50 S alleles at one locus (Pearson 1983). Dominant or co-dominant interactions may exist between any pair of alleles at this locus, and these relationships may differ between the anthers and the stigmas. The diploid tapetum is believed to be the source of the incompatibility phenotype in the pollen of species with sporophytically controlled SI systems, and the site of pollen inhibition is the stigma (Brewbaker 1957, Frankel and Galun 1977). Polyploidy has no effect on the function of this type of SI system (MacKay 1977).

In gametophytically controlled SI systems the pollen phenotype is determined by the haploid pollen genome and no dominance is therefore possible. In the pistils of plants with this type of SI system, alleles also function independantly (Frankel and Galun 1977). Gametophytic SI systems may be controlled by a single locus or by two or more loci acting in a complementary fashion (Lundqvist 1954, Osterbye 1975). The site of pollen inhibition in the gametophytically controlled species is usually the style (Brewbaker 1957), and colchicine-induced polyploidy generally disrupts the functioning of this SI system (MacKay 1977).

The major obstacle to the use of SI as a breeding tool has been the development of inbred lines. Techniques such as the use of mentor pollen (Pandey 1977), irradiation of pollen (Frankel and Galun 1977), thermally or electrically

aided pollination (Roggen et. al. 1972, Roggen and Van Dijk 1976, Ronald and Ascher 1975), removal of the stigmatic surface (Frankel and Galun 1977), or the application of chemicals such as cycloheximide (Ferran and Wallace 1977) or hexane (Ockendon 1978) have been used to circumvent the SI response, but are not practical on a field scale (Frankel and Galun 1977). Pollination of immature buds has proven the most consistantly reliable method and is the standard against which all others are judged (Roggen et. al. 1972).

Although exceptions exist, particularly in the multi-loci gametophytic species (Frankel and Galun 1977), pollen of gametophytic and sporophytic species may be distinguished based on the time of the division of the generative nucleus, whether before or after anthesis. In general, mature pollen of gametophytic species such as those in the Rosaceae is binucleate and pollen of sporophytic species is trinucleate (Brewbaker 1957,1967).

With very few exceptions the type of SI system is conserved within a botanical family (Frankel and Galun 1977). As all known species in the Rosaceae have a gametophytically controlled single locus SI system (Brewbaker 1957), this would be the type of system expected in P. fruticosa.

2.4 INDUCED MUTATIONS

Mutation induction is a tool available to plant breeders to increase the amount of genetic variability at their disposal (Micke and Donini 1982). This may be the only method of inducing variability in apomictic or sterile crops (Doorenbos and Karper 1975, Van Harten 1982).

In seed propagated crops such as cereals, induced mutations have been successful in improving several polygenic characters - increasing adaptibility, yield, earliness, seed size and seed protein. Lodging resistance has been improved through reductions in plant height and increases in straw strength, and induced mutations have also resulted in improved disease resistance (Micke and Donini 1982).

Ornamental crops are often highly heterozygous and vegetatively propagated and are therefore more difficult to improve through conventional breeding techniques. As novelty in appearance is often of value, the most desirable mutants are also the most easily detectable (Van Harten 1982). Due to the high degree of heterozygosity, recessive mutations may be more easily detectable in these crops. This is an advantage as most mutations are to recessive allelic forms and therefore may not otherwise be detectable without sexual recombination (North 1979).

Mutations may be induced by exposure of meristematic cells to ionizing radiation or chemical mutagens. Chemical

mutagens are less effective than ionizing radiation, although relatively inexpensive and easy to apply (North 1979, Nybom 1980). The most commonly used chemical mutagen is ethyl methane sulphonate (EMS). Chemical mutagens can cause changes in the nucleotides of the DNA through tautomeric shifts and the introduction of base analogs, thus causing copy errors, or by changes in nucleotide structure such as deaminations or hydroxylations (Strickberger 1985). These changes result in point mutations.

Ionizing radiation is short wavelength (less than 100 nm), high energy electromagnetic radiation. Due to the high energy of this type of radiation it is capable of penetrating tissues and producng ions by colliding with atoms and displacing electrons (Strickberger 1985). There are several types of ionizing radiation such as UV-, X-, and gamma rays, alpha and beta particles, protons and neutrons. These different types of radiation differ in energy and thus in penetrating and ionizing ability (Briggs 1970). Ionizing radiation may cause point mutations or chromosomal mutations such as deletions or translocations (Strickberger 1985), and may therefore be used to induce recombination between non-homologous genomes (Sears 1956).

Ionizing radiation may affect the genetic material of the cell directly through nucleotide alteration or chromosome breakage, or it may have an indirect effect through the ionizing of water and oxygen to form peroxides and superoxides

(Konzak et. al. 1970). For this reason the response of a tissue to ionizing radiation is partly dependent on the water and oxygen status of that tissue. Konzak et. al. (1970) state that in order to minimize radiation induced damage and thus maximize the yield of mutations, oxygen should be excluded from the irradiation chamber.

Temperature and mitotic index are also important factors, as cell division is required to propagate a mutation (Strickberger 1985). As the number of mutations increases linearly with radiation dosage, a single "hit" on a chromosome will cause a mutation. This is known as the target hypothesis (Strickberger 1985). Consequently the larger the nuclear volume (the target), the greater the probability of a "hit" (Konzak et. al. 1970). Sparrow et. al. (1968) investigated the radiosensitivity of 28 species and demonstrated an inverse relationship between radiosensitivity and Interphase Chromosome Volume (ICV). They predicted an LD-50 for species in the Rosaceae of 3.0 to 5.9 kR (2.6 to 5.1 krad).

Dormant scions or rooted cuttings are the most commonly irradiated plant parts in vegetatively propagated plants, and since radiation depresses vigor of the plant material, after treatment the plants should be placed under conditions which maximize cell division and growth (Nybom 1970). After reestablishment of the plant, growth of lateral bud primordia and adventitious buds should be encouraged in order to

detect mutations which are more likely to occur in undifferentiated cells (Bauer 1957, Bowen et. al. 1962, Broertjes 1972, Lapins 1965, Van Harten 1982). Bauer (1957) cut back irradiated black currant (Ribes nigrum) plants to the ground annually for three years and noted the appearance of new mutant shoots after each period of regrowth.

Repeated treatment of plant material has been used to produce groups of color mutants (Broertjes et. al. 1980, De Loose 1979). Successive use of two or more mutagens can increase both the frequency and spectrum of mutations (Broertjes 1976).

Induced mutations have been used to increase the genetic variability of a crop (Bauer 1957) and to increase disease resistance (McIntosh and Lapins 1966), but the most common uses of mutagenic treatment in horticultural species have been to induce flower mutations (Broertjes 1972, 1976, Broertjes et. al. 1980, De Loose 1979, Doorenbos and Karper 1975), and compact growth habit (Lapins 1962, 1965, Visser et. al. 1971). Changes in petal number have also been noted (Nybom 1970). Davidson (1986) recommended the application of mutation breeding techniques to P. fruticosa in order to increase petal number beyond fifteen.

Chapter III

MATERIALS AND METHODS

3.1 FLORAL MORPHOGENESIS

To determine the origin of extra petals, the development of flowers with and without extra petals was examined. As P. fruticosa 'Orange Whisper' has a constant petal number of five, and P. fruticosa 'Snowbird' has a relatively stable petal number of fifteen over most of the season, these two cultivars were used for preliminary morphological observations and comparisons. P. fruticosa 'Yellowbird' and other plants with intermediate petal numbers were observed later to determine the sequence in which the extra petals appear.

The continuous flowering habit of P. fruticosa allows the collection of shoot apices at all stages from vegetative to mature flowers at any time during the flowering period. Median and transverse sections of shoot apices were examined with the light microscope to determine the histological indicators of the onset of flowering. Shoot apices were also examined with the scanning electron microscope (SEM) to determine the pattern of initiation and differentiation of the floral organs. For consistency, only the primary flower of the inflorescence was examined.

Samples for examination with the light microscope were fixed in formalin-acetic acid-alcohol (FAA), dehydrated in an ethanol-butanol series (Johansen 1940) and embedded in TissuePrep paraffin. Serial sections were cut at 8 um, mounted on slides with Haupt's adhesive (Johansen 1940), and stained with Safranin O and Fast Green FCF according to the schedule in Appendix D for general histological analysis (Clark 1981) using a Leitz Ortholux microscope at 50-400X.

Samples for scanning electron microscopy were dissected under a Wild M3B stereomicroscope at 80X and fixed for 24h in 3% glutaraldehyde in a 0.1M sodium phosphate buffer (pH 6.8) followed by post-fixation in 1% osmium tetroxide in the same buffer for 16h. These operations were conducted at 4oC. These samples were dehydrated in an ethanol-acetone series (Appendix E), critical point dried in carbon dioxide, mounted on stubs with Electro Daz 416, and sputter coated with gold using a Balzer sputter coater. Samples were viewed with a Cambridge Stereo Scan Mk.IIA SEM at 10KV.

3.1.1 Quantitative Analysis

Floral organ numbers were analysed statistically to determine any quantitative relationships among the various floral organs. Petals, petalloid stamens, stamens, and pistils were counted weekly on five flowers each of existing F_1 hybrids between P. fruticosa 'Yellowbird' (UM7901) and three other cultivars: P. fruticosa 'Orange Whisper' (UM7904),

P.f. 'Pink Whisper' (UM7911), and P.f. 'Snowbird' (UM8102). Preliminary correlation analysis of these data revealed a relationship between petal number and stamen number. Backcross progeny families from these F_1 plants and their parents which contained over twenty individuals were analysed to verify this relationship. Floral organs from up to five flowers on each plant were counted weekly. Mean petal number and mean stamen number for each plant were analysed using the Statistical Analysis System (SAS) Analysis of Variance (ANOVA) and General Linear Models (GLM) procedures.

3.2 INHERITANCE OF PETAL NUMBER

The model for the inheritance of petal number proposed by Davidson (1986) was tested with a backcrossing program. Existing F_1 hybrids between P. fruticosa 'Yellowbird' (UM7901) and three other cultivars, P.fruticosa 'Orange Whisper' (UM7904), P. fruticosa 'Pink Whisper' (UM7911), and P. fruticosa 'Snowbird' (UM8102) in each of the three petal number classes defined by Davidson (1986) were backcrossed as pollen parents to both parents. The plants and the crosses made are shown in Table 1.

Flower buds were chosen for pollination in which the calyx had split but which had not yet opened. The primary flower of the inflorescence was emasculated and pollinated immediately. All other buds of the inflorescence were removed. Pollination was effected by the use of a camel

TABLE 1

Number of Backcrosses Completed

Plant Number	Mean Petal Number	Number of Backcrosses Maternal Parent	Number of Backcrosses Paternal Parent
(YB X OW)#4	5	60	53
(YB X OW)#5	14	55	67
(YB X OW)#44	14	53	66
(YB X OW)#112	8	53	57
(YB X PW)#19	5	47	46
(YB X PW)#30	8	60	55
(YB X PW)#93	13	55	50
(PW X YB)#100	5	51	47
(YB X SB)#15	5	64	60
(YB X SB)#46	10	53	53
(YB X SB)#47	15	55	51
(YB X SB)#50	6	54	62
(SB X YB)#21	8	51	51
(SB X YB)#32	5	55	62
(SB X YB)#79	15	50	52

OW = P. fruticosa 'Orange Whisper'
PW = P. fruticosa 'Pink Whisper'
SB = P. fruticosa 'Snowbird'
YB = P. fruticosa 'Yellowbird'

hair brush inoculated with pollen collected the previous day. Anthers were collected prior to anthesis and stored overnight to release their pollen which was used or discarded the next day. After pollination the flowers were covered with glassine bags to prevent uncontrolled pollination. Between crosses all emasculating and pollinating tools were sterilized in 95% ethanol to prevent contamination. All crossing was completed in June and July of 1986. Mature fruit was harvested approximately six weeks after pollination. Ripeness was visually determined by the change in color from immature green seed to mature brown seed.

The seed was manually extracted with forceps, counted, and sown in Terra-lite Redi-earth in 8cm. X 8cm. plastic cells as soon as practicable after harvesting. Seed which was not sown immediately was stored in envelopes on the laboratory bench. Seed was germinated in four lots sown on Oct. 1, Dec. 3, 1986, and Jan. 5 and Feb. 5, 1987 in a germination cabinet under a 16h. photoperiod at 25/20 C (day/night). Up to 300 seeds per cross (depending on the amount of seed obtained) from crosses 1 to 25 were sown on the first date and from crosses 26 to 46 on the second date (see Appendix A for the pedigree of numbered crosses). Additional seed, if available, was germinated on the two later dates to attain a target population of 100 progeny plants per cross. The actual numbers of seed and progeny seedlings are given in Appendix B. After 30 days, seedlings were transplanted into Spencer-Lemaire rootrainers (4cm X 4cm X 12 cm) containing a pasteurized 2:1:1 soil mix (soil:sand:peat), and grown in a growth room under a 16h photoperiod at 25/20 C. After another month the seedlings were moved to a greenhouse and held under natural light until field planting on May 4-6 1987.

The seedlings were planted in plots of four plants each in a completely randomized design as shown in Appendix C. Plants were spaced 30cm apart in the plots, and the plots were arranged in rows spaced 1.5m apart. Petals, petalloid stamens and stamens on up to five flowers per plant were

counted weekly on all flowering plants. The number of flowers counted was limited by the number of flowers on the plant and the time available. The plots were kept well irrigated for maximum expression of extra petals.

3.2.1 Reciprocal Differences

Davidson (1986) noted differences in reciprocal crosses between P. f. 'Snowbird' and P. f. 'Yellowbird'. In order to confirm the existence of such differences these two cultivars were intercrossed with 100 repetitions in each direction. Crossing techniques were as previously described. To further examine these differences F_1 hybrids from both reciprocal families involving these parents were used in the backcrossing program as both pollen and seed parents. The crosses made with the F_1 hybrids as seed parents are shown in Table 2. Seed and progeny were handled as above, with the exception that the target population was the maximum number of seedlings possible from the amount of seed available.

TABLE 2

Crosses made to test for reciprocal differences between P. f. 'Snowbird' and P. f. 'Yellowbird'

Seed Parent Family	Seed Parent Family Plant Number	Number of Crosses Pollen Parent YB	Number of Crosses Pollen Parent SB
YB X SB	15	39	39
	46	52	50
	47	29	30
	50	43	41
SB X YB	21	50	51
	32	4	2
	79	50	51

3.3 SELF-INCOMPATIBILITY

3.3.1 Pollen Cytology

Self-incompatibility is a breeding barrier in P. fruticosa and was expected to interfere with seed set in the backcross program. Pollen cytology may be used as an indicator of the type of SI system operating in a species. To determine the pollen cytology of P. fruticosa, anthers were collected from open flowers prior to anthesis, squashed in glacial acetic acid, cleared with chloral hydrate, and stained with hematoxylin as described by Kindiger and Beckett (1985). The stained pollen preparations were examined under the light microscope at 630X to determine their cytological state (i.e. binucleate or trinucleate).

3.3.2 Genetics of the Self-Incompatibility System

A program of controlled crosses was used to determine the genetics of the self-incompatibility system. P. f. 'Grandiflora' and P. f. 'Maaneleyi' have demonstrated absolute self-incompatibility (Davidson, 1986) and were therefore used in this part of the study. Existing reciprocal F_1 hybrids between these parents were intercrossed in a complete diallel design and the resulting seed was tested for emergence and compared to the emergence of open pollinated seed collected from the same plants on a seedlings-per-flower basis. Crossing, seed collecting and germination procedures were as previously described. The parent plants used in this part of the project were:

P. f. 'Maaneleyi' X P. f. 'Grandiflora'

Plant Number 1C, 9C, and 15C.

P. f. 'Grandiflora' X P. f. 'Maaneleyi'

Plant Number 1, 11, and 12.

Ten repetitions of each cross were made, for a total of 360 crosses, and ten open-pollinated flowers from each plant were tested, or a total of 60 open-pollinated flowers.

3.4 IRRADIATION OF CUTTINGS

Davidson (1986) suggested that induced mutagenesis may be an appropriate method of increasing the genetic variability in petal number, and possibly increasing the maximum number of petals beyond 15. Rooted cuttings were therefore irradiated

in an attempt to induce a mutation resulting in an increase in petal number. Greenwood cuttings were taken from vigorous shoots and rooted in 1:1 (peat moss and vermiculite) in an enclosed chamber with bottom heat. Cuttings were allowed to root for 30 days prior to irradiation. In order to determine the LD-50 for this species, ten rooted cuttings of P. f. 'Snowbird' were exposed to 0, 2, 4, 5, 6, 8, and 10 krad of gamma radiation from a Gammacell 220 with a Co-60 source emitting 120 krad/hr on 6/7/86. These cuttings were placed in a mist chamber for one week to assist their recovery and then transferred to a bench in the greenhouse where they were observed one month later for survival. The LD-50 was estimated to be 5 krad.

Twenty-five rooted cuttings of each of P. f. 'Orange Whisper', P. f. 'Pink Whisper', P. f. 'Snowbird', and P. f. 'Yellowbird' were irradiated at 4, 5, and 6 krad on 10/8/86. These cuttings were handled as previously and compared to 25 cuttings which were treated in an identical manner, but which had not been exposed to radiation.

The surviving cuttings were held over the winter in the greenhouse under the same conditions as the seedlings from the genetic part of the study, with the exception that they were planted in 10 cm. plastic pots. They were observed for survival and mutations, and planted in the field the following spring according to the planting plan in Appendix C, where they were further observed for mutations.

Chapter IV

RESULTS AND DISCUSSION

4.1 ONTOGENY OF EXTRA PETALS

4.1.1 Inflorescence Development

The growth habit of P. fruticosa is sympodial and is characterized by repeated growth flushes over the entire growing season. Each shoot is determinate in that it produces a terminal cymose inflorescence. Meristems arising in the axils of certain foliage leaves develop into new shoots shortly after their inception. The duration of the vegetative period prior to inflorescence formation is variable. Nevertheless, the growth pattern ensures that inflorescences are initiated continuously throughout the growing season. Secondary inflorescence branches develop in the axils of the two last formed foliage leaves (Fig. 1), each bearing a terminal flower and two new foliage leaves which produce tertiary inflorescence branches from their axils and so on. Foliage leaves in the inflorescence may have fewer leaflets than leaves on the vegetative portion of the shoot, but are still compound. Such indeterminate growth of the inflorescence may result in an inflorescence with five or more orders of branching. The continuous flowering habit of P. fruticosa results from the continuous initiation of inflorescences and their indeterminate growth habit.

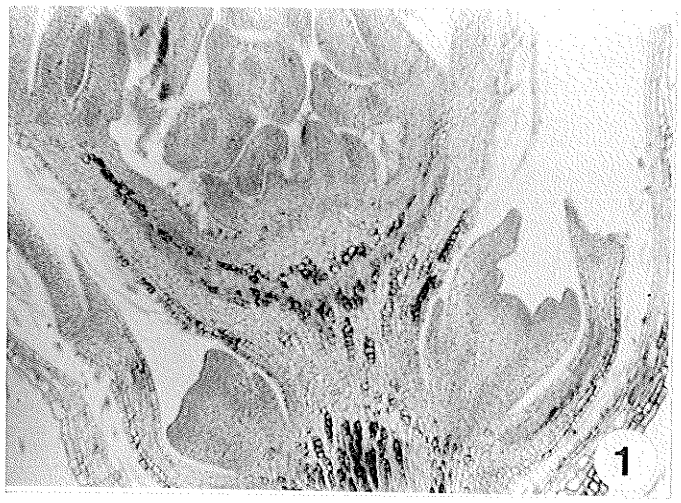


Figure 1. Longitudinal section showing secondary inflorescence branches in axils of two uppermost foliage leaves. x 75.

Each flower develops without a discernible pause from initiation to seed maturation, and thus all stages of floral development may be observed at any time during the growing season. The aesthetic value of the flowers may be enhanced by the presence of extra petals (Fig.2).

The genera Fragaria (strawberry) and Potentilla are closely related in that intergeneric hybridization is possible (Asker 1970, Jelenkovic et. al. 1984, MacFarlane Smith and Jones 1985). Despite a basic similarity in inflorescence structure, there are some important differences. In F. x ananassa Jahn and Dana (1970) found up to four orders of branching in the inflorescence prior to the onset of dormancy. All floral-organ primordia were present in the flower buds at this time and the sepals had elongated to enclose the buds, but neither differentiation of floral organs nor elongation of the inflorescence branches had occured. They concluded that inflorescence development was influenced by competition for space in the overwintering shoot apex. This is not the case in P. fruticosa. The immediate elongation of inflorescence branches eliminates any spatial constraints on development and thus allows more orders of branching and the development of a more symmetrical inflorescence than in F. x ananassa. Floral shoot apices of P. fruticosa do not appear to overwinter as a period of vegetative growth is required in the spring before reproductive structures are observed, but further reasearch is required to confirm this.

Figure 2. Single (left) and double (right) Flowers of P. fruticosa 'Snowbird'. x 3.

Inflorescence branches in F. x ananassa are initiated in the axils of two bracts which correspond to the last two foliage leaves in P. fruticosa, although much reduced in form. In the latter, the two foliage leaves on each inflorescence branch resemble the leaves formed during vegetative growth rather than bracts as found in F. x ananassa. This blend of vegetative and floral components in the inflorescence of P. fruticosa is an indicator of the indeterminate growth pattern of the cyme.

4.1.2 Floral Morphogenesis

The flower of P. fruticosa is perigynous and is composed of an inner and an outer calyx, each with five sepals, a corolla with a typical complement of five petals, a variable number of stamens (10-50), and a highly variable number of pistils (20-120). As noted above, the flowers are arranged in an indeterminate cyme.

Prior to conversion, the terminal vegetative shoot apical meritem is dome-shaped and leaf primordia are initiated in a typical helical pattern (Fig. 3). From the time of inception the primordia are crescent-shaped (P_1, Fig. 3). The flanks develop into stipules (S) which grow laterally to enclose the apex (cf. Figs. 3 and 5). The upper region of each primordium develops five lobes and will differentiate into a compound leaf with five leaflets (P_3). There is histological evidence for the initiation of a lateral bud in

the axil of P_2 (arrows, Fig. 4). First, the cells in the axil have a darkly staining meristematic appearance. Secondly, the beginning of an arcuate zone of elongate cells, the shell zone, is evident between the flanks of the apex and the incipient bud primordium. The development of a shell zone of cells is often associated with axillary bud initiation (Remphrey and Steeves 1984).

The conversion of the apex to the reproductive state is signalled by a broadening and flattening of the apex (Figs. 5 and 6). The last-formed foliage leaves, now present, are identical to all other foliage leaves on the shoot (cf. Figs. 3 and 5). Histologically, the apex is composed of a densely staining mantle over a vacuolated core (Fig. 6), as compared to the more homogeneous vegetative apex (cf. Fig. 4). The incipient bud meristem which will develop into the first secondary inflorescence branch is evident in the axil of the penultimate foliage-leaf primordium (see also Fig. 1). The broadening of the apex continues until it's diameter clearly exceeds that of the peduncle (Figs. 7-10). Elongation of the peduncle begins and elevates the apex above the stipules of the last two foliage leaves (Figs. 8 and 9).

The first floral organs to be initiated are five inner calyx primordia which develop at the periphery of the apex (Fig. 7). Their phyllotaxy is similar to that of the foliage leaves. The time interval between the inception

Figure 3. Terminal vegetative apex with three youngest leaf primordia (P_1 P_2 P_3). Note helical phyllotaxy and lateral growth of stipules (S) to enclose apex. x 150.

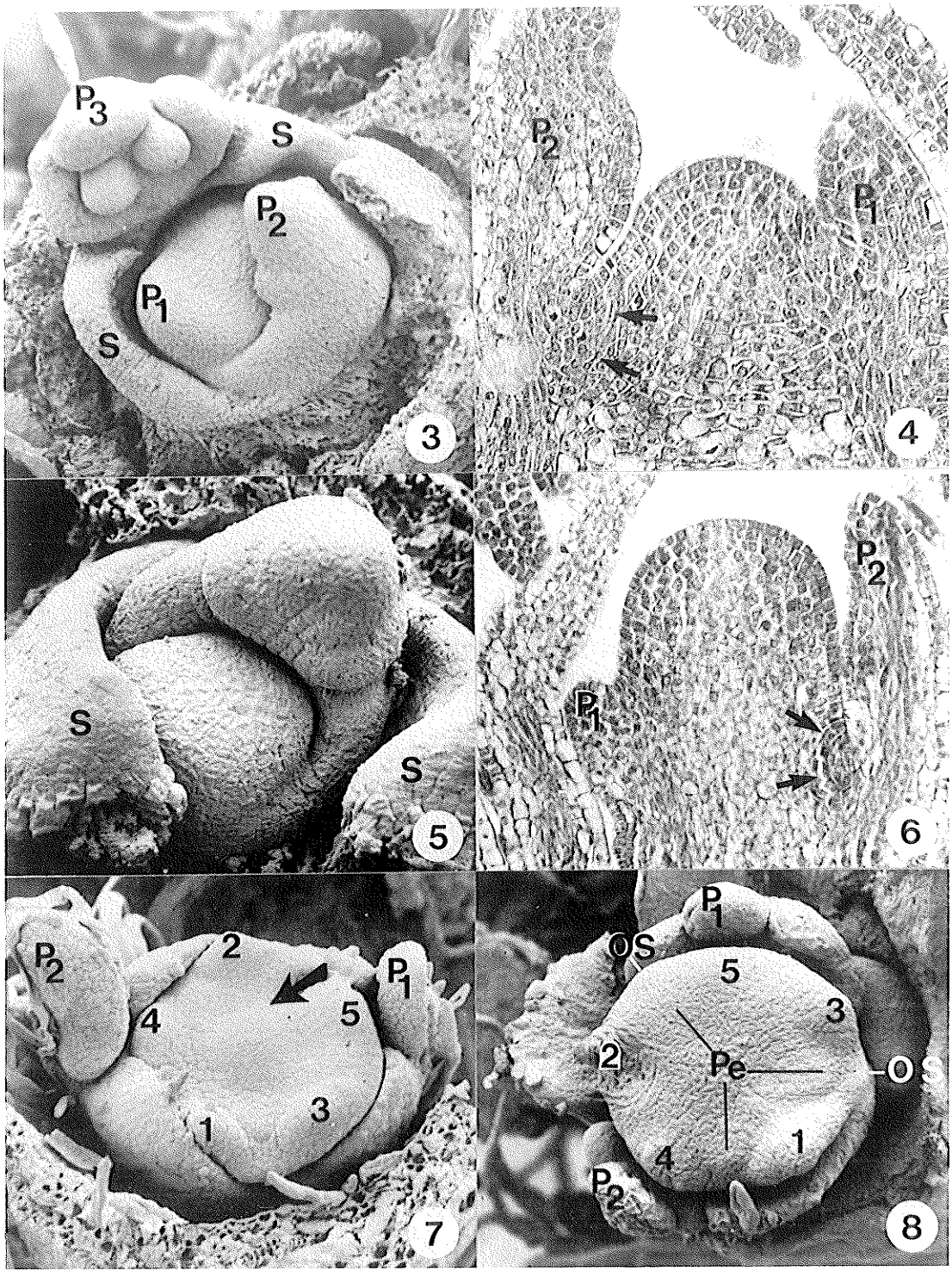
Figure 4. Median longitudinal section of terminal vegetative apex. Arrows indicate shell-like zone of cells delimiting incipient vegetative axillary bud. x 300.

Figure 5. Terminal apex converting to the floral state with penultimate leaf primordium removed leaving stipules (S). Note broadening and flattening of the apex. x 375.

Figure 6. Median longitudinal section of converting apex showing densely cytoplasmic mantle over vacuolate core. Arrows indicate shell-like zone of cells associated with axillary bud development. x 337.

Figure 7. Early floral apex with penultimate (P_2) and last-formed (P_1) leaf primordia. Inner calyx primordia (1 - 5) are numbered in order of inception (i.e., 5 is most recent). Arrow indicates reduced apical dome. x 200.

Figure 8. Slightly later floral apex that that shown in Figure 7. Petal (Pe) initiation is commencing. Outer sepal primordia (OS) are clearly visible opposite petal primordia. x 200.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8

of successive floral-organ primordia is much less than that of foliage-leaf primordia. The members of whorls of floral organs are thus initiated almost simultaneously. In contrast to the crescent-shaped foliage-leaf primordia, floral-organ primordia are essentially isodiametric at inception (Fig. 9). However the shape of the first floral organs, the sepals, represents a transitional shape (Figs. 7 to 9). After the initiation of the inner calyx the apical dome is much reduced in size (Fig. 7).

The petal and outer calyx primordia are initiated at sites between adjacent inner calyx primordia (IS, Fig. 8). The petals are initiated simultaneous with or just prior to the outer sepals. The initiation of these primordia follows the same helical pattern as previous primordia. Concomitant with their inception, ridges form between adjacent petal primordia at the periphery of the apex. This results in a continuous pentagonal ridge with the petal primordia located at the vertices (Fig. 9). In the mature flower the petals and stamens are borne along this ridge (Fig. 15), henceforth referred to as the androecial ridge. The carpels will arise inside this ridge from the central apical dome.

Sattler (1973) described floral development in Fragaria vesca, a species in which the occurrence of extra petals has been reported (Meyer 1966). Although floral development in this species is essentially similar to that in P. fruticosa, differences exist in the inception of the androecium.

Figure 9. Floral apex showing five incipient petal primordia (Pe) connected by the pentagonal androecial ridge (arrows). Inner sepals (IS) are starting to elongate vertically. x 171.

Figure 10. Median longitudinal section of a stage similar to that in Figure 9 showing vertical growth of inner sepals (IS) and outwards growth of outer sepals (OS). Petal primordia (Pe) and androecial ridge (AR) surround apical dome. Note cytoplasmic mantle over vacuolate core. x 180.

Figure 11. Developing flower bud showing stamen initiation (St) on androecial ridge adjacent to putative petal primordia (Pe) at each vertex of the pentagon. x 150.

Figure 12. Later stage of flower bud development showing stamen initiation proceeding along androecial ridge (arrows). Calyx is elongating to enclose flower bud. x 150.

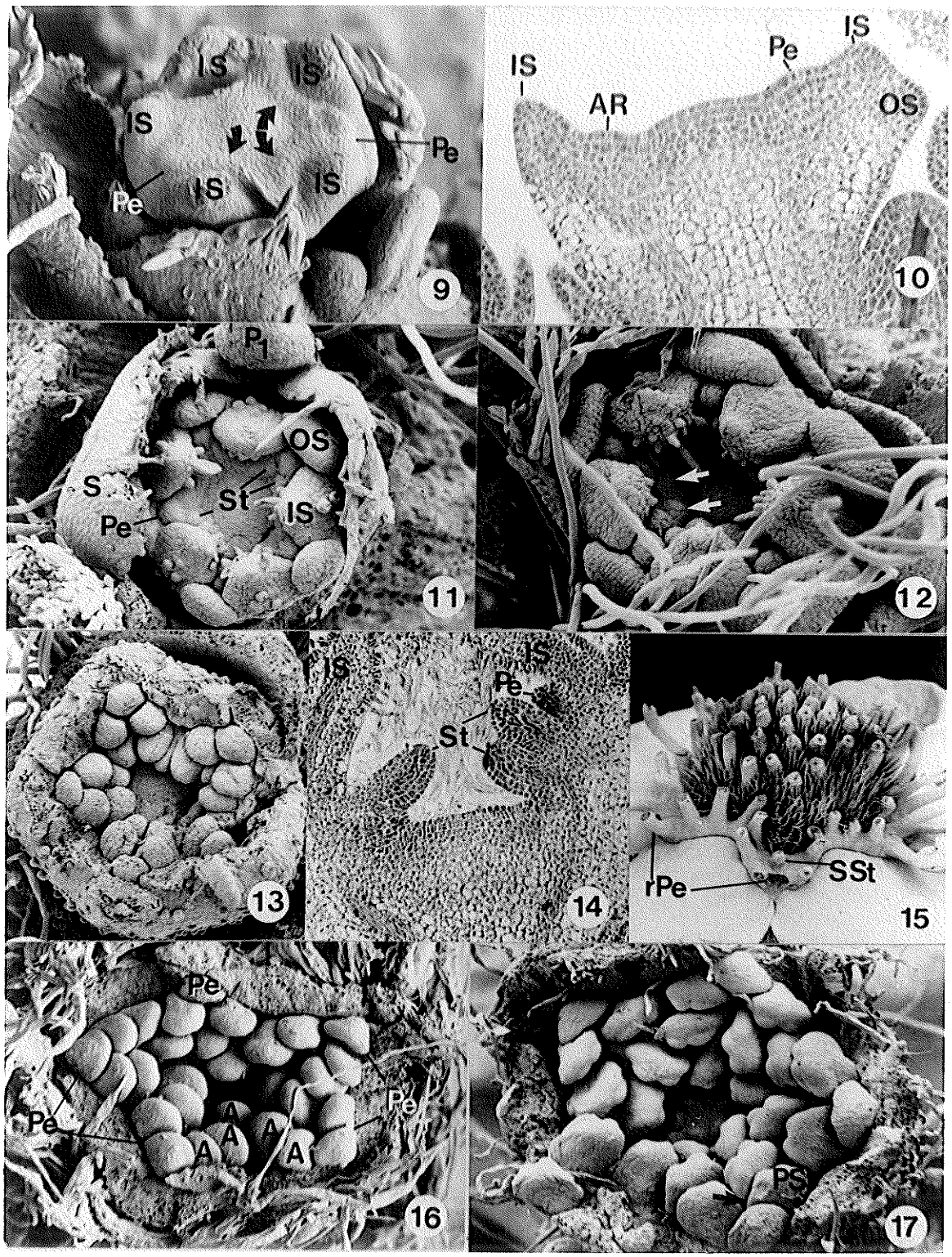
Figure 13. Flower bud with indistinguishable petal and stamen primordia along androecial ridge. Most of the calyx has been removed. x 110.

Figure 14. Median longitudinal section of a stage similar to Figure 13 showing elevation of petals (Pe) and stamens (St) on the outer portion of the androecial ridge to form the hypanthium. Inner sepals (IS) enclose the developing flower bud. x 160.

Figure 15. Mature open flower with petals (rPe) and anthers removed to show a supernumerary stamen (SSt) adaxial to a petal. The two petals indicated are separated by more than five stamens on the ridge, indicating the variability in stamen number which can occur. x 11.

Figure 16. Earliest stage in which stamens and petals can be distinguished in a five-petalled flower. Note lobing of anthers (A) and flattening of petals (Pe). x 85.

Figure 17. Five-petalled flower with at least one petalloid stamen (PS). Note marginal growth of anther (arrow). Carpel initiation is proceeding on the apex. x 85.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In F. vesca, five elongate androecial primordia are found in locations corresponding to the androecial ridge in P. fruticosa, but unlike the latter these are formed after and are not continuous with the petal primordia.

Concurrent with the establishment of the androecial ridge the inner calyx (IS) members elongate vertically. In contrast, the outer calyx (OS) members initially grow outward before growing vertically (Fig. 10). The calyx then rapidly grows to enclose the developing flower (Figs.11 and 12). This is similar to the pattern found in F. vesca (Sattler,1973). During this period the stamen primordia are initiated on the androecial ridge. Initiation proceeds from the vertices of the ridge (Fig. 11) toward the center of each side (Fig. 12). Typically, five putative stamen primordia are initiated on each arm of the ridge, for a total of about 25. Additional primordia (SSt) may arise on the ridge and on the adaxial side of each ridge vertex (Fig. 15). Petal and stamen primordia are indistinguishable at this point so that a large number of primordia with a similar appearance occur along the ridge (Fig. 13). Intercalary growth of the region of the receptacle underlying the androecial ridge elevates the developing calyx, corolla and androecium above the level of the apex to form the hypanthium (Fig. 14).

This pattern differs from the development of the androecium in F. vesca (Sattler 1973) in several respects. In F.

vesca, each of the five elongate androecial primordia produces three stamens, where in P. fruticosa each arm of the androecial ridge produces approximately five stamens, and perhaps more (Fig. 15). A further difference is that in F. vesca five antepetalous stamens are always formed, while in P. fruticosa although up to five are possible, this number is rarely achieved (see Figs. 15, 20, and 21).

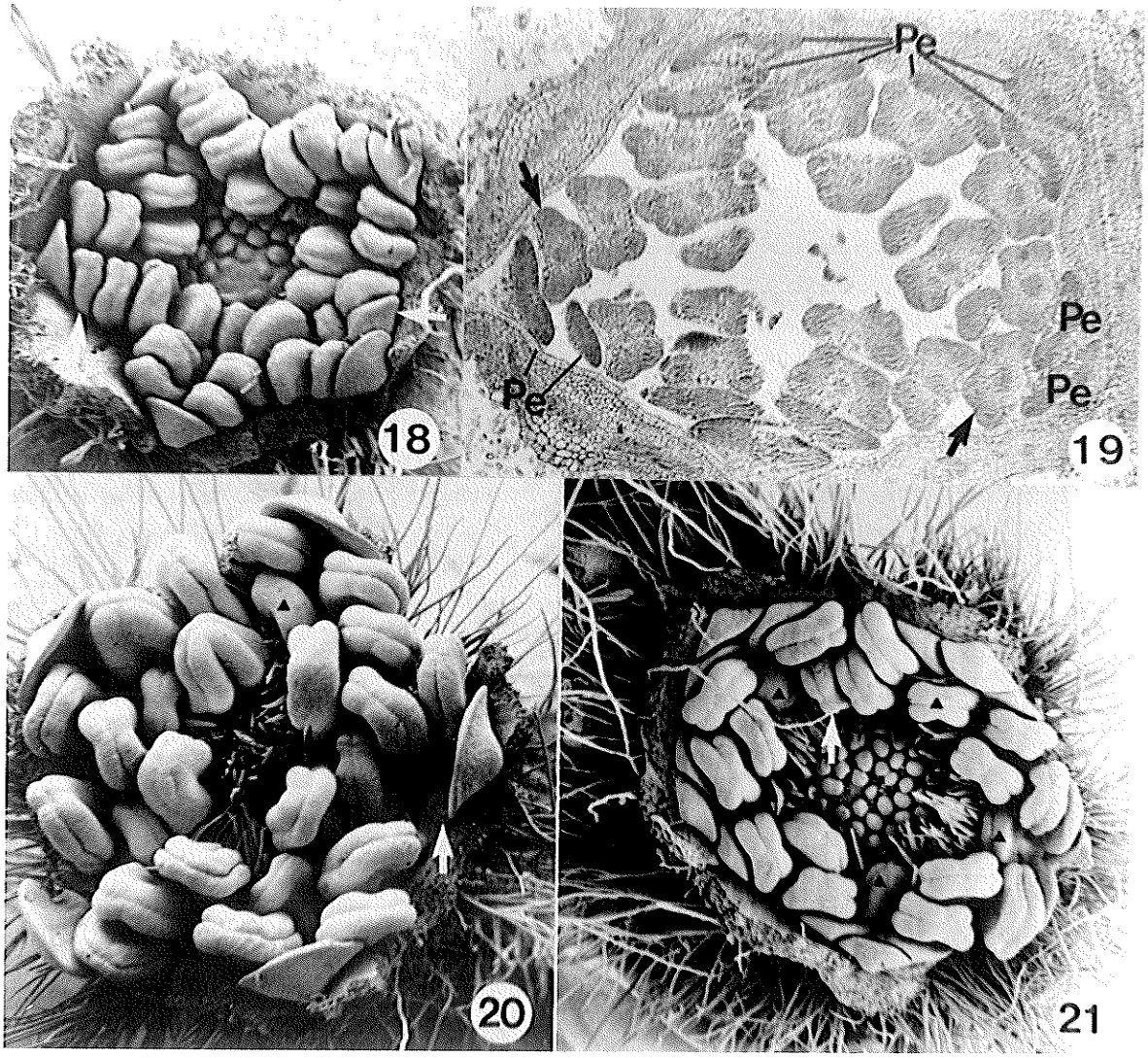
Concurrent with acropetal initiation and development of carpel primordia, the stamens and petals begin to differentiate. Primordia destined to become petals begin to show marginal growth and lobed anthers become visible on putative stamen primordia (Fig. 16). The five primordia at the vertices of the androecial ridge always develop into petals. The rest of the primordia on the ridge typically develop anthers and differentiate into stamens. Meiosis occurs in the anthers as carpel initiation is completed (i.e. stage illustrated in Fig. 18), and the petals then expand to ultimately enclose the mature flower bud. Intercalary growth of stamen filaments and petals then forces the flower to open. The development of the flower is therefore continuous from it's inception. The stigmas are receptive from the time of filament elongation and remain so for at least a day after the flower opens, depending on the environmental conditions. Anthesis occurs soon after the flower opens.

Figure 18. Differentiation of petals and stamens and initiation of carpels nearing completion. Several stamens have a petalloid appearance (arrows). A large number of stamens (total of 34) are present. x 50.

Figure 19. Transverse section of a flower bud at the level of the floral organs at a slightly more advanced stage than Figure 18. Note that all but two of the positions adjacent to the petals at the vertices are occupied by additional petals (Pe). The remaining two stamen primordia (arrows) of these positions are the most recently initiated. x 75.

Figure 20. Fully formed flower bud of P. f. 'Snowbird' with five petals, 25 stamens on the ridge, and one antepetalous stamen (▲). Arrow indicates site of broken stamen. x 35.

Figure 21. Fully formed double flower bud of P. f. 'Snowbird' with 15 petals, one extra stamen on the ridge (arrow), and four antepetalous stamens (▲). x 35.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4.1.3 Origin of Extra Petals

4.1.3.1 Morphological Observations

Observation of mature flowers indicated the presence of structures showing characteristics of both petals and stamens (Fig. 22). Such structures vary in form from nearly normal appearing stamens with petalloid outgrowths of the anther and/or filament, to nearly normal appearing petals with rudimentary anthers. The presence and appearance of these petalloid stamens suggests that extra petals in P. fruticosa may develop from modified stamens. Some of these petalloid stamens are dissected to give the appearance of two extra petals rather than only one. This species therefore shows both IIIa and Ia types of double flowers according to the classification system of Reynolds and Tampion (1983).

Developing flowers of genotypes producing single and double flowers are indistinguishable until the time of differentiation of the petals and stamens. In genotypes characterized by the presence of extra petals, any or all of the primordia adjacent to those at the vertices may take on petalloid characteristics to a variable degree. The number of primordia which acquire such petalloid characteristics is dependant on genetic and environmental factors.

Figure 22. Petalloid stamens from P. fruticosa 'Yellowbird' indicating the variability in morphology. Anthers are indicated by arrows. x 5.


22

Once most of the carpels have been initiated, distinctly flattened petals and lobed anthers are clearly distinguishable (Figs. 17 and 18). Although their final form (see Fig. 22) is unknown at this stage, the beginning of the development of petalloid stamens can be seen in the marginal growth of certain anthers and/or filaments (arrows, Fig. 18). At the slightly earlier stage illustrated in Fig. 17, such marginal growth may also be detectable.

The differentiation of floral organs, excluding the calyx, appears to occur almost simultaneously. Nevertheless, the location of anomolous floral organs such as petalloid stamens and extra petals gives an indication that there is a pattern to the sequence of differentiation. The presumptive stamen primordia which acquire petalloid characteristics do so in a sequence which parallels the phyllotactic pattern of their inception. The first anomalous organs to become recognizable are the oldest primordia in the putative stamen positions. For example, in Fig. 19 the primordia to the right of the petals at the vertices of the androecial ridge have also differentiated into petal-like structures. Those primordia to the left have differentiated into petal-like structures at the three older positions, but not at the two younger positions (arrows, Fig. 19). The result is a flower with 13 petals.

In the mature (fully formed) bud of a single flower, the petals at the vertices of the androecial ridge are separated

by approximately five stamens (Fig. 20). These correspond to the five primordia which were initiated along each side of the ridge (cf. Fig.13). In a flower with fifteen petals, those at the vertices are also separated by a group of five floral organs, but this group is composed of three stamens flanked by two petals (Fig. 21). This results in five groups of three petals. Therefore, these observations indicate that the two petals adjacent to those at the vertices are in positions which would otherwise be occupied by stamens, and suggests that extra petals in this species develop at the expense of stamens.

It is clear that petals and stamens in P. fruticosa arise from the same zone of the floral apex, that is the androecial ridge (Fig. 15). The first-formed putative petal primordia at the vertices of the ridge are radially the most distant from the apex. The next primordia are initiated adjacent to the first, that is at the next most distant sites from the apex, and initiation proceeds towards the centers of the arms of the ridge, radially the least distant from the apex. This pattern is similar to that found in Fragaria vesca (Sattler 1973). In those P. fruticosa plants with extra petals, the conversion of primordia from stamens to petals occurs in the same sequence as primordium initiation (see Fig. 19), suggesting that such conversion may be a function of distance from the apex, age of the primordium, or both. It appears that when primordia are being deter-

mined as stamens, certain older putative stamen primordia are exposed to a petal forming stimulus such that they become either petalloid stamens or extra petals. Such a pattern suggests that either the determination of stamens occurs basipetally as in Lythrum salicaria (Cheung and Sattler 1967), or that older primordia were already influenced by a petal forming stimulus early in their development. The observation that anther development precedes any visible sign of petalloid outgrowths of such stamen primordia would tend to support the former interpretation, although clarification of this point would require physiological and analytical investigation.

4.1.3.2 Quantitative Analysis of Floral Organs

The morphological observations indicated that extra petals are found in sites otherwise occupied by stamens. It should therefore be possible to confirm such a relationship by analysis of petal and stamen numbers. Preliminary analysis of the backcross population revealed a relatively weak but significant correlation ($r=-0.44$, $P<0.0001$), showing a tendency for stamen number to decrease as petal number increased. In explaining the source of the variability, one-way analysis of variance of mean stamen and petal numbers revealed significant differences among families. The families were therefore analysed separately to reduce the variability due to the genetic component.

Another source of variation which has the potential to mask a possible inverse relationship between petal number and stamen number was developmental plasticity resulting from environmental influences. That portion of the variability due to environmental factors was measured as the variation over the different sampling dates. The first flowers produced by a plant had fewer floral organs than later formed flowers. Such a pattern is illustrated for P. fruticosa 'Orange Whisper' (Fig.23) and P. fruticosa 'Snowbird' Fig. 24) and is similar to the pattern found by Robertson (1984). Although there were no significant differences at the 0.05 significance level for either mean petal number or mean stamen number between sampling dates (P=0.25 and 0.07 respectively), the significance levels suggest that stamen number is a more variable character than petal number (see also Figs. 23 and 24). The interaction between families and dates was not significant, indicating that different genotypes responded similarly to the environment.

The production of extra petals in P. fruticosa is under genetic control (Davidson 1986) although subject to plastic modification by environmental influences, particularly in relation to temperature and moisture stress (Robertson 1984). The present investigation revealed considerable variability in both petal number and stamen number.

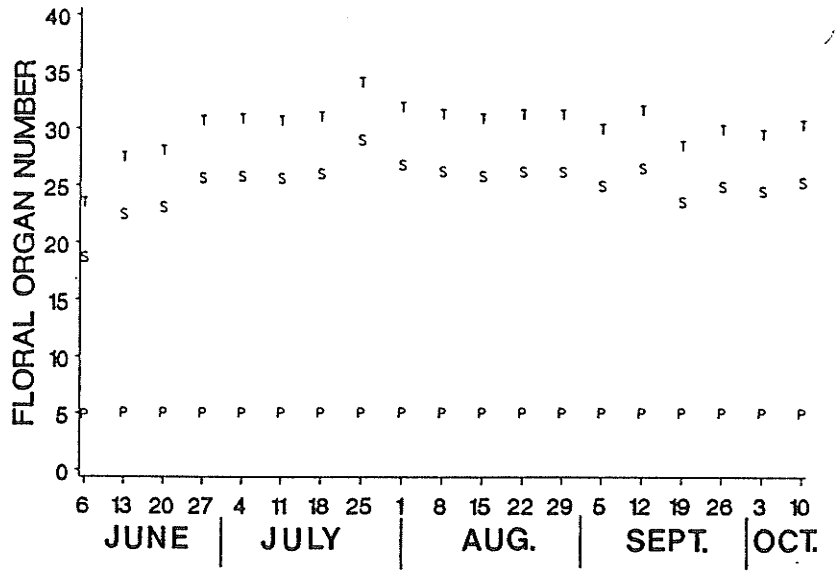

Figure 23. Variation in petal number (P), stamen number (S), and total (T) for the single flowered P. f. 'Orange Whisper' over the growing season.

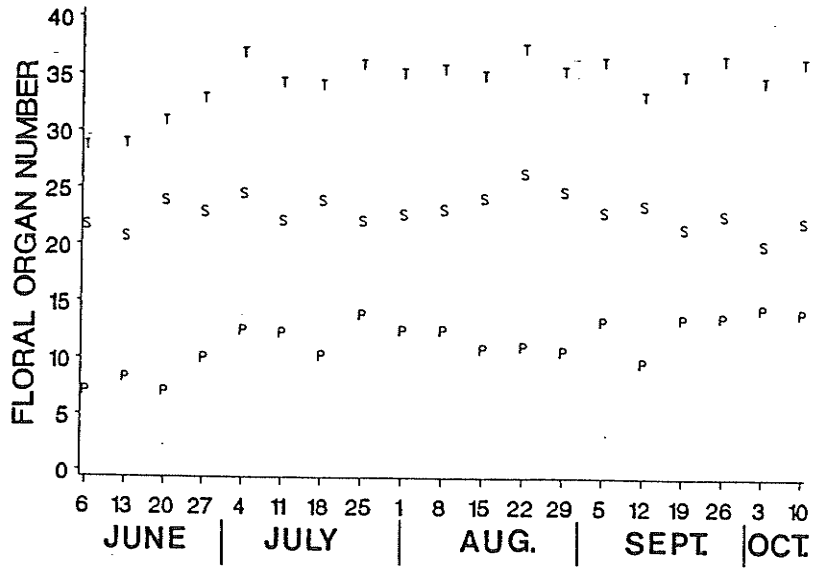

Figure 24. Variation in petal number (P), stamen number (S), and total (T) for the double flowered P. f. 'Snowbird' over the growing season.

Stamen number is the more variable of the two because there are several potential sources of variation. In addition to variation resulting from the inverse relationship between stamen and petal numbers, extra stamens may be initiated independently of petals, either on the androecial ridge or in positions adaxial to it's vertices (Fig. 15). Increases in petal number however may occur only at the expense of stamen formation as no extra petals were observed in positions other than those typically occupied by stamens.

To control the variability, data from families containing over 20 individuals were collected for a three week period between 17 Aug. and 9 Sept. 1987, and subjected to regression analysis. Although there was still considerable unexplained variability, the coefficients of determination (r^2) for each family were improved (Table 3). Moreover, there was a consistent inverse relationship between the two variables for each family.

Despite the high level of plasticity evident in this species, it was possible to demonstrate an inverse relationship between petal and stamen numbers both morphologically and statistically. A significant linear regression was noted in all families. The slopes of the regression lines varied from -0.67 to -0.96, indicating that in those flowers where stamens adjacent to existing petals acquire petalloid characteristics, there is an attendant increase in the number of stamen primordia initiated on the androecial ridge,

TABLE 3

Linear Regression Statistics for Stamens vs. Petals of P. fruticosa in Order of Decreasing r^2 Values

Family	Regression Coefficient	r^2	p (1)
(YB X SB)#15 X SB	-0.90	0.62	0.02
SB X (SB X YB)#32	-0.78	0.51	<0.0001
(YB X SB)#50 X YB	-0.89	0.38	<0.0001
(SB X YB)#79 X YB	-0.80	0.37	0.001
SB X (YB X SB)#15	-0.82	0.32	0.02
YB X (YB X SB)#50	-0.76	0.30	0.009
SB X (YB X SB)#50	-0.96	0.28	0.02
YB X SB	-0.71	0.26	<0.0001
YB X (SB X YB)#21	-0.67	0.24	<0.0001
Yellowbird (YB)	-0.89	0.68	0.01
Snowbird (SB)	-0.84	0.11	0.04

(1) Significance level of regression.

although the converse was not necessarily the case. However, the slopes indicate that this increased stamen initiation only partially compensated for the reduction in stamen number resulting from the conversion of some stamens to petals. In the absence of any such compensation, the regression coefficients would be expected to be -1.0, in other words one stamen replaced by one petal. Since some compensation exists, the expression of genes controlling flower doubleness appears to occur early enough in floral development to permit the initiation of more primordia on the androecial ridge. The time of gene action may therefore be during primordium initiation, rather than at the time of differentiation, however further histochemical and physio-

logical studies are required to confirm such an interpretation. Research into the environmental effects on the total number of primordia on the androecial ridge may also be beneficial. Another possibility is that the genes conferring doubleness have a pleiotropic effect increasing the total number of primordia on the androecial ridge, however such an increase is not sufficient to fully compensate for the decrease in stamen number due to the conversion of putative stamen primordia to petals.

The results of the present investigation indicate that extra petals in P. fruticosa are derived from modified stamen primordia. This conclusion was inferred from the positional relationships of the petals and stamens on the androecial ridge and substantiated by quantitative analysis of petal and stamen numbers. Similarities in location and arrangement of primordia suggest that the corolla and androecium represent a continuum on the androecial ridge, and the fate of any primordium is dependant on it's age and position on the ridge, within the limits set by genetic and environmental factors.

4.2 INHERITANCE OF PETAL NUMBER

4.2.1 Backcross Program

4.2.1.1 Existing F_1 Plants

In developing his proposed model, Davidson (1986) sorted flowers of P. fruticosa into three classes based on multi-

ples of the basic petal number of five. Single flowered plants consistently had five petals. Plants with extra petals fell into two classes with six to ten petals and eleven to fifteen petals. For this part of the study, any member of the corolla or androecium with a petalloid appearance was considered to be a petal. This definition included the petalloid stamens encountered in the morphological study.

The petal numbers of several of the F_1 plants used as parents in the backcross program differed from the petal numbers assigned by Davidson (1986). The observed petal numbers from the 1986 growing season are shown in Table 4. It is not uncommon for the petal number of a plant with double flowers to require two or more growing seasons to stabilize at a level which reflects the genotype of the plant (Reynolds and Tampion 1983). Environmental factors, notably temperature and moisture stresses, may also influence petal number. Robertson (1984) found that stressed plants had fewer petals than unstressed plants. These phenomena are also expected to complicate the analysis of the seedling population generated by the backcross program as it was analysed in the first growing season, and misclassification of some genotypically double plants as single is to be expected. In the case of the F_1 plants used as parents this resulted in a change in the petal number class assigned to three plants, (YB X OW)#5, (YB X PW)#19 and (YB X SB)#15.

TABLE 4

Mean Petal Numbers of F_1 Plants and Parental Controls

Plant Number	Mean Petal Number (1985) (Davidson 1986)	Mean Petal Number (1986)	Standard Error of Mean (1986)
(YB X OW)#4	5	5.0	0.02
(YB X OW)#5	14	7.5	0.23
(YB X OW)#44	14	11.0	0.33
(YB X OW)#112	8	7.4	0.28
(YB X PW)#19	5	6.2	0.19
(YB X PW)#30	8	6.1	0.20
(YB X PW)#93	13	12.7	0.27
(PW X YB)#100	5	5.0	0.01
(YB X SB)#15	5	5.5	0.09
(YB X SB)#46	10	7.1	0.24
(YB X SB)#47	15	14.5	0.12
(YB X SB)#50	6	6.8	0.20
(SB X YB)#21	8	9.0	0.29
(SB X YB)#32	5	5.0	0.00
(SB X YB)#79	15	15.3	0.17
Parental controls			
Orange Whisper		5.0	0.01
Pink Whisper		5.5	0.09
Snowbird		11.3	0.32
Yellowbird		8.5	0.28

4.2.1.2 Backcross Progeny

The numbers of seed and progeny resulting from the crosses made in 1986 are shown in Table 5. Out of a total of 2445 progeny from these crosses, 1044 or 42.7% flowered in 1987. Since less than half of the progeny flowered in the first year, the analysis of the population is necessarily based on the assumption that there is no correlation between petal number and flowering at an early age. This may not be the case as Robertson (1984) reported that vigorous plants tended to produce flowers with a greater number of petals. Rey-

nolds and Tampion (1983) also associate increased vigor with an increase in petal number.

4.2.1.3 Segregation for Petal Number

The petal numbers of the parental plants used as controls increased in 1987. This was not unexpected as moisture stress is known to decrease petal number in P. fruticosa (Robertson 1984), and the progeny plots were kept well irrigated to allow maximum expression of the double flowering character. While P. fruticosa 'Orange Whisper' remained constant at 5.00 ± 0.01 petals, P. fruticosa 'Pink Whisper' had 5.89 ± 0.61 petals, P. fruticosa 'Yellowbird' had 10.91 ± 0.67 petals, and P. fruticosa 'Snowbird had 14.27 ± 0.36 petals. For this reason the petal number classes were adjusted to 5, 6-11, and over 11 petals. This did not affect the differentiation between single flowers and those with extra petals, and the segregation for the presence or absence of extra petals is shown in Table 6. This change would not have affected the original model. The hypothetical genotypes in Table 6 were developed based on the possible genotypes for each plant according to the model proposed by Davidson (1986). Theoretical ratios for each possible genotype were compared to the observed segregation ratios, and the genotypes with the best fit were assigned to the hybrids. In the two cases where the actual ratio differed significantly from the tested ratio, the differences were due to a greater number of plants with extra petals than was expected.

TABLE 5

Progeny from Backcrosses Made in 1986

Cross	Number of Crosses Attempted	Total Seed	Seed Sown	Total Progeny	Flowering Progeny
F_1 Plants used as Pollen Parents					
YB X (YB X OW)#4	60	116	116	42	30
OW X (YB X OW)#4	53	122	122	10	10
YB X (YB X OW)#5	55	118	118	53	30
OW X (YB X OW)#5	67	90	90	6	3
YB X (YB X OW)#44	53	483	483	177	103
OW X (YB X OW)#44	66	73	73	7	5
YB X (YB X OW)#112	53	164	164	54	35
OW X (YB X OW)#112	57	23	23	1	1
YB X (YB X PW)#19	47	30	30	0	0
PW X (YB X PW)#19	46	1	1	0	0
YB X (YB X PW)#30	60	182	182	77	50
PW X (YB X PW)#30	55	66	66	11	10
YB X (YB X PW)#93	55	248	248	44	23
PW X (YB X PW)#93	60	87	87	14	9
YB X (PW X YB)#100	51	9	9	0	0
PW X (PW X YB)#100	47	39	39	8	5
YB X (YB X SB)#15	64	168	168	22	13
SB X (YB X SB)#15	60	398	398	49	30
YB X (YB X SB)#46	53	205	205	64	26
SB X (YB X SB)#46	53	224	224	44	11
YB X (YB X SB)#47	55	622	622	104	31
SB X (YB X SB)#47	51	211	211	91	40
YB X (YB X SB)#50	54	204	204	48	28
SB X (YB X SB)#50	62	707	707	222	37
SB X (SB X YB)#21	51	678	678	230	104
YB X (SB X YB)#21	51	566	566	48	12
SB X (SB X YB)#32	55	722	450	21	14
YB X (SB X YB)#32	62	144	144	101	69
SB X (SB X YB)#79	50	1572	650	95	37
YB X (SB X YB)#79	52	853	400	54	14
F_1 Plants used as Seed Parents					
(YB X SB)#15 X YB	39	332	332	58	33
(YB X SB)#15 X SB	39	659	659	36	11
(YB X SB)#46 X YB	52	202	202	41	24
(YB X SB)#46 X SB	50	491	400	101	26
(YB X SB)#47 X YB	29	251	251	100	13
(YB X SB)#47 X SB	30	163	163	22	14
(YB X SB)#50 X YB	43	373	373	69	42
(YB X SB)#50 X SB	41	601	500	73	37
(SB X YB)#21 X SB	51	1011	500	72	34
(SB X YB)#21 X YB	50	1008	500	46	9
(SB X YB)#32 X SB	2	4	4	0	0
(SB X YB)#32 X YB	4	2	2	0	0
(SB X YB)#79 X SB	51	841	500	76	38
(SB X YB)#79 X YB	50	819	500	54	13

In the case of PW X (YB X PW #93), this was probably due to the low number (9) of progeny which flowered. The plant YB X OW #44 gave anomolous results for the single/double segregation. Approximately twice the expected proportion of double flowers was observed. Due to the low number (5) of progeny obtained the genotype proposed for PW X YB #100 cannot be considered reliable.

Where the expected ratio consisted of all double flowered progeny (0:1), the X^2 test is inappropriate. In many of these crosses, up to 15% of the progeny had only five petals. This may have been due to the low expressivity of the genes governing doubleness in the first flowers produced by a plant. Robertson (1984) noted that petal numbers in P. fruticosa were lower than normal in the first few flowers to appear in the spring, and Reynolds and Tampion (1983) cite several instances where petal number may require several seasons to stabilize at the level specified by the genotype of the plant. This phenomenon has been encountered in this study as noted above with regard to the classification of the F_1 plants by Davidson (1986). In many cases in the backcross population the first flowers to appear were the only flowers available for observation as the plants did not begin to flower until late in the growing season. Plants in the backcross population with a lower petal number than expected may therefore be anomolous, and may stabilize at a higher petal number in subsequent seasons.

TABLE 6

Segregation for the Presence or Absence of Extra Petals

Hypothetical Genotype of Hybrid	Cross	Petals 5	Petals >5	Test Ratio	X^2	Probability of X^2	Remarks
D_1d_1 D_2__ DmDm	PW X (YB X PW)#100	3	2	3:1	0.06	.90 - .70	2
D_1d_1 D_2__ DmDm	YB X (PW X YB)#100	0	0				2
D_1d_1 D_2d_2 Dmdm	SB X (SB X YB)#32	18	51	3:5	3.36	.10 - .05	
D_1d_1 D_2d_2 Dmdm	YB X (SB X YB)#32	8	6	3:5	1.54	.30 - .20	
D_1d_1 D_2d_2 Dmdm	(SB X YB)#32 X SB	0	0				2
D_1d_1 D_2d_2 Dmdm	(SB X YB)#32 X YB	0	0				2
D_1d_1 D_2d_2 dmdm	OW X (YB X OW)#4	6	4	3:1	0.53	.50 - .30	
D_1d_1 D_2d_2 dmdm	YB X (YB X OW)#4	7	23	3:5	2.00	.20 - .10	
D_1d_1 d_2d_2 Dmdm	OW X (YB X OW)#5	3	0	1:1	2.00	.20 - .10	
D_1d_1 d_2d_2 Dmdm	YB X (YB X OW)#5	3	27	1:3	2.84	.10 - .05	
D_1d_1 d_2d_2 Dmdm	OW X (YB X OW)#112	0	1	1:1	--	---------	2
D_1d_1 d_2d_2 Dmdm	YB X (YB X OW)#112	6	30	1:3	1.33	.30 - .20	
D_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#15	4	26	0:1		---------	3
D_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#15	5	8	1:3	0.64	.50 - .30	
D_1d_1 d_2d_2 Dmdm	(YB X SB)#15 X SB	1	11	0:1		---------	3
D_1d_1 d_2d_2 Dmdm	(YB X SB)#15 X YB	12	21	1:3	1.71	.20 - .10	
d_1d_1 D_2__ Dm__	PW X (YB X PW)#19	0	0				2
d_1d_1 D_2__ Dm__	YB X (YB X PW)#19	0	0				2
d_1d_1 D_2__ Dmdm	PW X (YB X PW)#30	5	5	1:1	0.00	1.0	
d_1d_1 D_2__ Dmdm	YB X (YB X PW)#30	8	42	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#46	1	10	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#46	4	22	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(YB X SB)#46 X SB	2	22	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(YB X SB)#46 X YB	2	22	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#50	10	27	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#50	5	23	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(YB X SB)#50 X SB	1	36	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(YB X SB)#50 X YB	4	38	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	SB X (SB X YB)#21	1	11	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	YB X (SB X YB)#21	16	96	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(SB X YB)#21 X SB	1	8	0:1		---------	3
d_1d_1 d_2d_2 Dmdm	(SB X YB)#21 X YB	2	32	0:1		---------	3
D_1d_1 d_2d_2 dmdm	OW X (YB X OW)#44	2	3	1:1	0.00	1.0	2
D_1d_1 d_2d_2 dmdm	YB X (YB X OW)#44	14	89	1:3	6.55	.05 - .01	1
d_1d_1 D_2__ dmdm	PW X (YB X PW)#93	0	9	1:1	7.10	.01 -.001	1
d_1d_1 D_2__ dmdm	YB X (YB X PW)#93	2	21	0:1		---------	3
d_1d_1 d_2d_2 dmdm	SB X (YB X SB)#47	0	40	0:1		---------	3
d_1d_1 d_2d_2 dmdm	YB X (YB X SB)#47	2	29	0:1		---------	3
d_1d_1 d_2d_2 dmdm	(YB X SB)#47 X SB	1	13	0:1		---------	3
d_1d_1 d_2d_2 dmdm	(YB X SB)#47 X YB	0	14	0:1		---------	3
d_1d_1 d_2d_2 dmdm	SB X (SB X YB)#79	2	11	0:1		---------	3
d_1d_1 d_2d_2 dmdm	YB X (SB X YB)#79	2	36	0:1		---------	3
d_1d_1 d_2d_2 dmdm	(SB X YB)#79 X SB	0	13	0:1		---------	3
d_1d_1 d_2d_2 dmdm	(SB X YB)#79 X YB	2	36	0:1		---------	3

$X^2_{05} = 3.84$

(1) X^2 is significant - reject hypothetical genotype.
(2) Insufficient progeny to analyse reliably.
(3) X^2 is inappropriate to test 0:1 ratio.

A similar problem with gene expressivity was encountered by Wilson (1987), dealing with filament color in cotton flowers.

The segregation for the two different classes of double flowers is shown in Table 7 and reflects the segregation at the Dm locus. Only the results for those crosses which yielded sufficient progeny to analyse are presented.

The crosses in which the observed ratio for the segregation into the two double flowered classes (6-11 and over 11 petals) differed significantly from the ratio expected on the basis of the model proposed by Davidson (1986) occurred in three groups. Group I contained (YB X OW)#5, (YB X OW)#112, and (YB X SB)#15, all with the genotype Dd dd Dmdm, and (YB X PW)#30 with the genotype dd Dd Dmdm. When crossed with P. fruticosa 'Yellowbird' this group should have yielded a 3:1 ratio for the two extra petal classes, however a 1:1 ratio was observed, resulting in an overall ratio of 2:3:3 (5 petals:6-11 petals:>11 petals) rather than 4:9:3 as expected. When (YB X OW)#5 and (YB X OW)#112 were crossed to P. fruticosa 'Orange Whisper' insufficient progeny were obtained to analyse. When (YB X PW)#30 was crossed to P. fruticosa 'Pink Whisper' the results fit a 3:1 ratio as expected, although only five double flowered plants were obtained. When YB X SB #15 was crossed to P. fruticosa 'Snowbird', a 1:1 ratio was obtained as expected.

TABLE 7

Segregation of Double Flowered Plants

Hypothetical Genotype of Hybrid	Cross	Petal Number 6-11	Petal Number >11	Test Ratio	X^2	Probability of X^2	Remarks
D_1d_1 D_2__ DmDm	PW X (PW X YB)#100	2	0	1:0	0.00	1.0	2
D_1d_1 D_2d_2 Dmdm	SB X (SB X YB)#32	28	23	1:1	0.31	.70 - .50	
D_1d_1 D_2d_2 Dmdm	YB X (SB X YB)#32	5	1	3:1	0.00	1.0	
D_1d_1 D_2d_2 dmdm	YB X (YB X OW)#4	12	11	1:1	0.00	1.0	
D_1d_1 d_2d_2 Dmdm	YB X (YB X OW)#5	11	16	3:1	15.12	<.001	1
D_1d_1 d_2d_2 Dmdm	YB X (YB X OW)#112	15	15	3:1	8.71	.01 -.001	1
D_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#15	15	11	1:1	0.35	.70 - .50	
D_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#15	4	4	3:1	1.50	.30 - .20	
D_1d_1 d_2d_2 Dmdm	(YB X SB)#15 X SB	5	6	1:1	0.00	1.0	
D_1d_1 d_2d_2 Dmdm	(YB X SB)#15 X YB	10	11	1:3	4.58	.05 - .01	1
d_1d_1 D_2__ Dmdm	YB X (YB X PW)#30	25	17	3:1	4.57	.05 - .01	1
d_1d_1 D_2__ Dmdm	PW X (YB X PW)#30	2	3	3:1	1.67	.20 - .10	
d_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#46	2	8	1:1	2.50	.20 - .10	
d_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#46	11	11	3:1	6.06	.05 - .01	1
d_1d_1 d_2d_2 Dmdm	(YB X SB)#46 X SB	11	11	1:1	0.00	1.0	
d_1d_1 d_2d_2 Dmdm	(YB X SB)#46 X YB	10	12	3:1	8.72	.01 -.001	1
d_1d_1 d_2d_2 Dmdm	SB X (YB X SB)#50	16	11	1:1	0.59	.50 - .30	
d_1d_1 d_2d_2 Dmdm	YB X (YB X SB)#50	11	12	3:1	7.66	.01 -.001	1
d_1d_1 d_2d_2 Dmdm	(YB X SB)#50 X SB	11	25	1:1	4.69	.05 - .01	1
d_1d_1 d_2d_2 Dmdm	(YB X SB)#50 X YB	18	20	3:1	8.98	.01 -.001	1
d_1d_1 d_2d_2 Dmdm	SB X (SB X YB)#21	7	4	1:1	0.36	.70 - .50	
d_1d_1 d_2d_2 Dmdm	YB X (SB X YB)#21	41	55	3:1	51.68	<.001	1
d_1d_1 d_2d_2 Dmdm	(SB X YB)#21 X SB	4	4	1:1	0.00	1.0	
d_1d_1 d_2d_2 Dmdm	(SB X YB)#21 X YB	14	18	3:1	15.04	<.001	1
D_1d_1 d_2d_2 dmdm	YB X (YB X OW)#44	39	50	1:1	1.12	.30 - .20	
d_1d_1 D_2__ dmdm	PW X (YB X PW)#93	4	5	1:1	0.00	1.0	
d_1d_1 D_2__ dmdm	YB X (YB X PW)#93	6	15	1:1	3.05	.10 - .05	
d_1d_1 d_2d_2 dmdm	SB X (YB X SB)#47	7	33	0:1		---	2
d_1d_1 d_2d_2 dmdm	YB X (YB X SB)#47	11	18	1:1	1.24	.30 - .20	
d_1d_1 d_2d_2 dmdm	(YB X SB)#47 X SB	1	13	0:1		---	2
d_1d_1 d_2d_2 dmdm	(YB X SB)#47 X YB	6	8	1:1	0.00	1.0	
d_1d_1 d_2d_2 dmdm	SB X (SB X YB)#79	1	10	0:1		---	2
d_1d_1 d_2d_2 dmdm	YB X (SB X YB)#79	7	29	1:1	12.25	<.001	1
d_1d_1 d_2d_2 dmdm	(SB X YB)#79 X SB	0	13	0:1		---	2
d_1d_1 d_2d_2 dmdm	(SB X YB)#79 X YB	7	29	1:1	12.25	<.001	1

$X^2_{05} = 3.84$

(1) X^2 is significant - reject hypothetical genotype.

(2) X^2 is inappropriate.

Group II contained (YB X SB)#46, (YB X SB)#50, and (SB X YB)#21, all with the genotype dd dd Dmdm. When crossed with P. fruticosa 'Yellowbird' as either pollen or seed parents, the model calls for an overall ratio of 0:3:1. The observed ratio, however, was 0:1:1. Furthermore, when crossed as seed parents to P. fruticosa 'Snowbird', the progeny from (YB X SB)#46 and (YB X SB)#21 fit a 0:1:1 ratio as expected, but the progeny from (YB X SB)#50 fit a 0:1:3 ratio. When crossed to P. fruticosa 'Snowbird' as pollen parents, the progeny from all three plants fit a 0:1:1 ratio as expected.

Group III contained SB X YB #79 with the genotype dd dd dmdm. When crossed with P.f. 'Yellowbird', a 0:1:1 ratio was expected from this plant, but the X^2 was significant due to an excess of plants in the higher petal number class. A 0:0:1 ratio was expected in crosses between this plant and P.f. 'Snowbird', and a reasonable fit to this ratio was obtained. The plant YB X SB #47 has the same genotype, and progeny from all crosses involving this plant segregated as expected.

Both groups I and II gave results when crossed with P. fruticosa 'Yellowbird' which were consistent with the Dm allele of the hybrid not being transmitted. It was therefore hypothesized that the modifier locus (Dm) was closely linked to the self-incompatibility locus (S). Such linkage could not have been incorporated by Davidson (1986) in the model as backcrosses would be required to detect it. This

linkage between Dm and S is also not the cause of the reciprocal differences noted by Davidson (1986) in crosses between P. fruticosa 'Yellowbird' and P. fruticosa 'Snowbird'. These two cultivars do not have any S alleles in common (Table 8). The Dm allele in plants in groups I and II came from P. fruticosa 'Yellowbird', and the dm allele from the other parent. Since P. fruticosa 'Snowbird' is homozygous dmdm (Davidson 1986), this is evident in its hybrids even without the evidence provided by linkage to the S locus. P.fruticosa 'Pink Whisper and P.fruticosa 'Orange Whisper' appear to have two S alleles in common,and to share one S allele with P. fruticosa 'Snowbird' (Davidson 1986). From the success in backcrossing hybrids involving these cultivars to P. fruticosa 'Yellowbird', it appears that P. fruticosa 'Pink Whisper' and P. fruticosa 'Orange Whisper' also have one S allele in common with P. fruticosa 'Yellowbird'. Proposed genotypes for these cultivars are given in Table 8.

The other crosses fit both the original and the modified model, with the exception of those crosses involving the two plants in group III. As no recombination was observed between the Dm and S loci, the increase in petal number due to the action of the dm allele could be a pleiotropic effect of a certain S allele.

The progeny from crosses of SB X YB #79 with P. fruticosa 'Yellowbird' had an excess of plants in the higher petal

number class. The observed segregation for both reciprocals fit a 0:1:3 ratio rather than a 0:1:1 ratio as expected. Davidson (1986) reported altered segregation in crosses between P. fruticosa 'Yellowbird' and P. fruticosa 'Snowbird' but his data indicated reciprocal differences involving the presence or absence of extra petals rather than altered segregation of the two extra petal classes. Davidson suggested that environmental or cytoplasmic factors may be involved, and P. fruticosa 'Snowbird' cytoplasm is involved in both cases. Other possible explanations suggested by Davidson were linkage to a pollen lethal gene, preferential fertilization, or selective elimination of zygotes, although all are rare. The difference in the loci involved suggests the additional possibility that a transposable element may be implicated in inactivating the Dm allele in this instance. Further research is required to determine the cause of the aberrant segregation of certain crosses involving P. fruticosa 'Snowbird'. The segregation for the two extra petal number classes based on the modified model is shown in Table 9. The cross (YB X SB)#46 X SB fit a 1:1 ratio rather than the predicted 3:1 ratio. This anomolous result could be due to the small population size (22) or to low expressivity of the recessive allele at the Dm locus.

TABLE 8

Genotypes of Parental Cultivars Based on Modified Model

Cultivar	Genotype
P.f. 'Orange Whisper'	DD Dd Dm-S*dm-S*
P.f. 'Pink Whisper'	Dd DD Dm-S*dm-S*
P.f. 'Snowbird'	Dd dd $dm-S_3dm-S_4$
P.f. 'Yellowbird'	dd Dd $Dm-S_1dm-S_2$

*'Pink Whisper' and 'Orange Whisper' appear to have the same genotype at the S locus, either S_1S_3, S_1S_4, S_2S_3, or S_2S_4

TABLE 9

Segregation of Double Flowers Based on Modified Model

Hypothetical Genotype of Hybrid	Cross	Petal Number 6-11	Petal Number >11	Test Ratio	X^2	Probability of X^2
D_1d_1 D_2__ Dm-S_1Dm-S_7	PW X (PW X YB)#100	2	0	1:0	0.00	1.0
D_1d D_2d_2 Dm-S_1dm-S*	SB X (SB X YB)#32	28	23	1:1	0.31	.70 - .50
D_1d_1 D_2d_2 Dm-S_1dm-S*	YB X (SB X YB)#32	5	1	3:1	0.00	1.0
D_1d_1 D_2d_2 dm-S_2dm-S_6	YB X (YB X OW)#4	12	11	1:1	0.00	1.0
D_1d_1 d_2d_2 Dm-S_1dm-S_6	YB X (YB X OW)#5	11	16	1:1	0.59	.50 - .30
D_1d_1 d_2d_2 Dm-S_1dm-S_6	YB X (YB X OW)#112	15	15	1:1	0.00	1.0
D_1d_1 d_2d_2 Dm-S_1dm-S*	SB X (YB X SB)#15	15	11	1:1	0.35	.70 - .50
D_1d_1 d_2d_2 Dm-S_1dm-S*	YB X (YB X SB)#15	4	4	1:1	0.00	1.0
D_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#15 X SB	5	6	1:3	1.48	.30 - .20
D_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#15 X YB	10	11	1:1	0.00	1.0
d_1d_1 D_2__ Dm-S_1dm-S_8	PW X (YB X PW)#30	2	3	3:1	1.67	.20 - .10
d_1d_1 D_2__ Dm-S_1dm-S_8	YB X (YB X PW)#30	25	17	1:1	1.52	.30 - .20
d_1d_1 d_2d_2 Dm-S_1dm-S*	SB X (YB X SB)#46	2	8	1:1	2.50	.20 - .10
d_1d_1 d_2d_2 Dm-S_1dm-S*	YB X (YB X SB)#46	11	11	1:1	0.00	1.0
d_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#46 X SB	11	11	1:3	6.06*	.05 - .01
d_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#46 X YB	10	12	1:1	0.00	1.0
d_1d_1 d_2d_2 Dm-S_1dm-S*	SB X (YB X SB)#50	16	11	1:1	0.59	.50 - .30
d_1d_1 d_2d_2 Dm-S_1dm-S*	YB X (YB X SB)#50	11	12	1:1	0.00	1.0
d_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#50 X SB	11	25	1:3	0.33	.70 - .50
d_1d_1 d_2d_2 Dm-S_1dm-S*	(YB X SB)#50 X YB	18	20	1:1	0.02	.90 - .70
d_1d_1 d_2d_2 Dm-S_1dm-S*	SB X (SB X YB)#21	7	4	1:1	0.36	.70 - .50
d_1d_1 d_2d_2 Dm-S_1dm-S*	YB X (SB X YB)#21	41	55	1:1	2.04	.20 - .10
d_1d_1 d_2d_2 Dm-S_1dm-S*	(SB X YB)#21 X SB	4	4	1:3	1.50	.30 - .20
d_1d_1 d_2d_2 Dm-S_1dm-S*	(SB X YB)#21 X YB	14	18	1:1	0.28	.70 - .50
D_1d_1 d_2d_2 dm-S_2dm-S_6	YB X (YB X OW)#44	39	50	1:1	1.12	.30 - .20
d_1d_1 D_2__ dm-S_2dm-S_8	PW X (YB X PW)#93	4	5	1:1	0.00	1.0
d_1d_1 D_2__ dm-S_2dm-S_8	YB X (YB X PW)#93	6	15	1:1	3.05	.10 - .05
d_1d_1 d_2d_2 dm-S_2dm-S*	SB X (YB X SB)#47	7	33	0:1		---
d_1d_1 d_2d_2 dm-S_2dm-S*	YB X (YB X SB)#47	11	18	1:1	1.24	.30 - .20
d_1d_1 d_2d_2 dm-S_2dm-S*	(YB X SB)#47 X SB	1	13	0:1		---
d_1d_1 d_2d_2 dm-S_2dm-S*	(YB X SB)#47 X YB	6	8	1:1	0.00	1.0
d_1d_1 d_2d_2 dm-S_2dm-S*	SB X (SB X YB)#79	1	10	0:1		---
d_1d_1 d_2d_2 dm-S_2dm-S*	YB X (SB X YB)#79	7	29	1:1	12.25*	<.001
d_1d_1 d_2d_2 dm-S_2dm-S*	(SB X YB)#79 X SB	0	13	0:1		---
d_1d_1 d_2d_2 dm-S_2dm-S*	(SB X YB)#79 X YB	7	29	1:1	12.25*	<.001

X^2_{05} = 3.84

S* may be either S_3 or S_4

In ten crosses plants with petal numbers exceeding 22 were obtained (Table 10). These extra petals are due to at least one additional recessive modifier gene (dm_2). In the cross (SB X YB)#79 X SB, a 3:1 ratio is observed for the 12-21 and >21 petal number classes, indicating that one gene may be segregating, but in several other crosses (i.e. YB X (YB X OW)#112, YB X (YB X SB)#47, (YB X SB)#47 X SB, YB X (SB X YB)#79, and (YB X SB)#50 X SB) a 15:1 ratio is observed, suggesting two duplicate genes. Further research is required to clarify the number of additional modifiers operating in this system.

TABLE 10

Extra Petals Due to the Action of the Dm_2 Locus

Cross	Plants with Petal Number 12-21	>21
YB X (YB X OW)#44	49	1
YB X (YB X OW)#112	14	1
YB X (YB X SB)#47	17	1
YB X (SB X YB)#21	53	2
YB X (SB X YB)#79	27	1
(YB X SB)#47 X YB	6	1
(YB X SB)#47 X SB	12	1
(YB X SB)#50 X SB	24	1
(SB X YB)#79 X SB	8	5
SB X YB	48	1

4.2.2 Reciprocal Differences

The reciprocal differences reported by Davidson (1986) in crosses between P. fruticosa 'Yellowbird' and P. fruticosa 'Snowbird' are not affected by the linkage of the S locus to the Dm locus as these two cultivars do not have any S alleles in common. Backcrosses of hybrids between these two cultivars to P. fruticosa 'Yellowbird' are not expected to show any reciprocal differences. However, such differences are expected in backcrosses of these hybrids to P. fruticosa 'Snowbird' as only hybrid pollen carrying the Dm allele will be functional on P. fruticosa 'Snowbird' stigmas due to the linkage of the Dm and S loci, and P. fruticosa 'Snowbird'pollen can carry only the dm allele. The homogeneity X^2's for those crosses where no reciprocal differences are expected are shown in Table 11. No significant differences were observed in crosses between P. fruticosa 'Yellowbird' and P. fruticosa 'Snowbird', or between P. fruticosa 'Yellowbird' and any of its hybrids with P. fruticosa 'Snowbird'. These results are therefore consistent with expectations, and indicate that the reciprocal differences noted by Davidson (1986) for crosses involving these two cultivars were anomolous.

TABLE 11

Homogeneity X^2's for Reciprocal Crosses Involving *P. fruticosa* 'Snowbird' and *P. fruticosa* 'Yellowbird'

Cross	5	6-10	>11	Test Ratio	X^2homo
YB X SB	16	34	48		
SB X YB	30	77	61	4:6:6	0.01
(YB X SB)#15 X YB	12	10	11		
YB X (YB X SB)#15	5	4	4	4:6:6	0.58
(YB X SB)#46 X YB	2	10	12		
YB X (YB X SB)#46	4	11	11	0:1:1	0.09
(YB X SB)#47 X YB	0	6	8		
YB X (YB X SB)#47	2	11	18	0:1:1	0.09
(YB X SB)#50 X YB	4	18	20		
YB X (YB X SB)#50	5	11	12	0:1:1	0.08
(SB X YB)#21 X YB	2	14	18		
YB X (SB X YB)#21	16	41	55	0:1:1	0.52
(SB X YB)#79 X YB	2	7	29		
YB X (SB X YB)#79	2	7	29	0:1:1	0.00

$X^2_{05} = 5.99$

4.3 SELF INCOMPATIBILITY

4.3.1 Pollen Cytology

To determine the number of nuclei in the pollen, anthers were examined prior to anthesis as described by Kindiger and Beckett (1985). The pollen of *P. fruticosa* was found to be binucleate (Fig. 25). Binucleate pollen is typical of species exhibiting homomorphic gametophytically controlled self-incompatibility systems (Brewbaker, 1957, 1967).

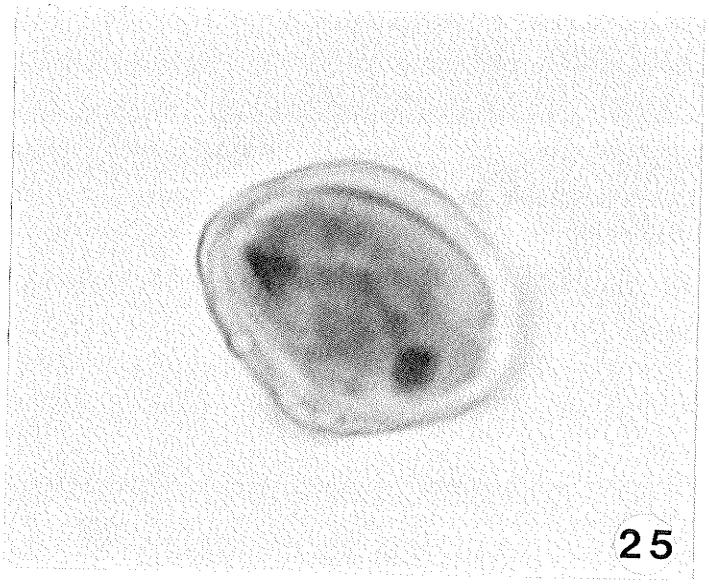

Figure 25. Binucleate pollen grain of P. fruticosa. x 2500.

4.3.2 Diallel Crosses

The results of the diallel crosses made to determine the inheritance of the self-incompatibility system operating in P. fruticosa are presented in Table 12.

TABLE 12

Mean Seed Set and Seedlings per Flower from Diallel Crosses of F_1 Hybrids between P. fruticosa 'Maaneleyi' and P. fruticosa 'Grandiflora'

Cross	Seed	Seedlings	Cross	Seed	Seedlings
1 OP	15.5	2.1	1C OP	4.9	1.3
1 X 1	0.0	0.0	1C X 1	3.9	3.0
1 X 11	6.5	1.4	1C X 11	0.0	0.0
1 X 12	5.3	1.2	1C X 12	3.9	0.7
1 X 1C	3.9	1.2	1C X 1C	0.0	0.0
1 X 9C	7.1	3.6	1C X 9C	1.0	0.4
1 X 15C	1.7	0.0	1C X 15C	0.5	0.2
11 OP	11.8	4.2	9C OP	17.1	6.2
11 X 1	0.5	0.0	9C X 1	13.0	10.1
11 X 11	0.0	0.0	9C X 11	15.0	6.8
11 X 12	0.4	0.0	9C X 12	16.4	3.8
11 X 1C	0.2	0.1	9C X 1C	4.8	2.5
11 X 9C	6.7	2.6	9C X 9C	0.0	0.0
11 X 15C	0.3	0.1	9C X 15C	5.0	4.5
12 OP	14.9	0.4	15C OP	7.7	3.6
12 X 11	2.6	0.7	15C X 1	0.7	0.0
12 X 11	0.0	0.0	15C X 11	0.0	0.0
12 X 12	0.0	0.0	15C X 12	0.2	0.2
12 X 1C	0.3	0.1	15C X 1C	0.8	0.4
12 X 9C	10.8	1.0	15C X 9C	1.4	1.3
12 X 15C	0.0	0.0	15C X 15C	0.0	0.0

Plant 1 = P. f.'Maaneleyi' X P.f.'Grandiflora' #1
Plant 11 = P. f.'Maaneleyi' X P.f.'Grandiflora' #11
Plant 12 = P. f.'Maaneleyi' X P.f.'Grandiflora' #12
Plant 1C = P. f.'Grandiflora' X P. f.'Maaneleyi' #1C
Plant 9C = P. f.'Grandiflora' X P. f.'Maaneleyi' #9C
Plant 15C = P. f.'Grandiflora' X P. f.'Maaneleyi' #15C

As a result of the seedling emergence shown in Table 12, crosses between the six F_1 plants were classified as either compatible (+) or incompatible (-) as shown in Table 13. The F_1 plants fell into four intra-incompatible, inter-compatible groups.

TABLE 13

Classification of Diallel Crosses

	15C	1	12	11	1C	9C
15C	-	-	+	-	+	+
1	-	-	+	+	+	+
12	-	+	-	-	+	+
11	+	-	-	-	+	+
1C	+	+	+	-	-	+
9C	+	+	+	+	+	-

\+ Compatible Cross
\- Incompatible Cross

Certain crosses did not fit the pattern, although in all such cases the results of the reciprocal crosses were as expected and enabled classification of the cross. The crosses 1C X 11, 15C X 11 and 12 X 15C set no seed, and although five seeds were obtained from the cross 11 X 1, no seedlings emerged. Pollen viability was not a factor in the failure of these crosses as the pollen which did not result in seed set in these cases did result in seed set on other plants on the same dates. For the same reason environmental factors or crossing technique do not appear to be implicated in the failure of these crosses.

The interpretation of the results of the diallel is that P. fruticosa 'Maaneleyi' and P. fruticosa 'Grandiflora' are both heterozygous at the self-incompatibility (S) locus, and have no S alleles in common. The proposed genotypes for P. fruticosa 'Maaneleyi', P. fruticosa 'Grandiflora', and the F_1 plants involved in the study are given in Table 14. The S alleles are designated Sa, Sb, Sc, and Sd to indicate that these may or may not be the same alleles found in the genetic study. Since no crosses were made between the two groups of plants, the relationships of the alleles found is unknown.

TABLE 14

Genotypes at the S Locus of Plants in the SI Study

Plant	Genotype
'Grandiflora'	Sa,Sb
'Maaneleyi'	Sc,Sd
15C, 1	Sa,Sc
12, 11	Sa,Sd
1C	Sb,Sc
9C	Sb,Sd

Based on the pollen cytology and the diallel crosses, the self-incompatibiity system operating in P. fruticosa is concluded to be controlled by a single multiallelic gene functioning gametophyticaly in the pollen.

4.4 IRRADIATION OF CUTTINGS

The survival of cuttings exposed to various doses of gamma radiation on 6/7/86 is shown in Table 15. The LD-50 for rooted cuttings of P. f. 'Snowbird' exposed to gamma radiation was estimated at 5 krad.

TABLE 15

Survival of Rooted Cuttings Irradiated on 6/7/86

Dose(krad)	% Survival after 30 days
2	60
3	90
4	60
5	20
6	30
8	40
10	40

Rooted cuttings of P. fruticosa 'Orange Whisper', P. fruticosa 'Pink Whisper', P. fruticosa 'Snowbird', and P. fruticosa 'Yellowbird' were irradiated at each of 0, 4, 5, and 6 krad on 10/8/86. At the highest dose, a reduction in vigor was noted in all cultivars. Smaller than normal leaves and shorter than normal internodes characterized these plants, although these effects were not persistent and had disappeared after six weeks. The resulting plants were planted in the field as previously noted and observed for mutations. No detectable mutations were observed in the field. Petal numbers of irradiated plants were comparable to non-irradiated control plants. The survival of these

plants is shown in Appendix F, and suggests that the LD_{50} may be 6 krad rather that 5 krad as previously stated.

These irradiated plants should be severely pruned each spring to induce adventitious buds which may show mutations, and should be interpollinated in order to detect any recessive mutations which may not be expressed in the original material.

Chapter V

GENERAL DISCUSSION

The sequence of initiation of primordia on the androecial ridge is a continuation of the acropetal helix established by the foliage leaf primordia and the inner calyx, although greatly compressed. As a result of the acropetal sequence of primordium initiation, the age of a primordium is related to its position, with the oldest primordia located at the vertices of the ridge and the youngest at the centers of the arms of the ridge. From the examination of flowers at various stages of development it is apparent that the extra petals in double flowers of P. fruticosa are the result of petallody of stamens. Petal and stamen primordia appear to form a continuum on the androecial ridge, and the developmental fate of any primordium is dependent on its age and/or position on the ridge. This effect is probably mediated by plant hormones.

In flowers with petalloid stamens the development of anthers on putative stamen primordia precedes any petalloid outgrowths of the primordium, suggesting that the determination of stamens occurs in a basipetal direction. This would be consistent with a chemical signal inducing stamen determination or limiting petal determination diffusing downwards

from the floral apex. The extent of this diffusion would be affected by the ammount of the substance produced and the duration of production.

Raman and Greyson (1977) found much higher levels of Gibberellic acid (GA) in double flowers of Nigella damascena than in single flowers. Sawhney (1983) found that the effect of GA on the number of floral organs of tomato could be duplicated by low temperatures. Robertson (1984) reported that high temperatures in the field reduced petal number in P. fruticosa. These results suggest that the chemical signal inducing stamen determination may be a GA inhibitor. Moisture stress and other stresses have been found to increase the production of GA inhibitors such as ethylene and abscisic acid (Salisbury and Ross 1985).

Hormone metabolism is known to be affected by environmental factors (Salisbury and Ross 1985), and could mediate the plasticity which characterizes floral morphogenesis in P. fruticosa. The variability in petal number within a genotype could thus be due to variation in the amount or the time of production of the signal inducing stamen determination. The observed low expressivity of the recessive alleles for extra petals could also be due to variable GA metabolism in floral apices of younger plants. Studies of hormonal balances and metabolism in the floral apex of single and double flowering plants at various stages of development would be useful to confirm such an interpretation.

An interpretation of the genetic model consistent with the above hypothesis is that each D locus codes for a gene product which induces the determination of stamens from the undifferentiated primordia on the androecial ridge (or arrests the determination of petals) after a specific cycle, with each cycle producing one whorl of five petals. This gene product may be an enzyme functioning in the synthesis of a GA inhibitor as discussed above.

In the case of the D_1 and D_2 loci, both gene products are required and the absence of either will result in the determination of an additional whorl of primordia as petals (Davidson 1986). The active agent may therefore be a dimer composed of two non-identical subunits (Strickberger 1985). The modifier genes could affect different whorls of primordia, based on primordium age and position relative to the apex, and function only in an environment such as may result from the absence of a gene product from a prior locus in the hierarchy.

The linkage intensity of the Dm and S loci was not determined in this study, and should be investigated. Another possible explanation for the apparent association of these loci is that rather than the two loci being tightly linked, the increase in petal number due to the action of the dm allele could be a pleiotropic effect of a certain S allele. It is not unknown for the S locus to affect floral morphology. For example, distyly in Primula is attributed to a

pleiotropic effect of the S locus (Frankel and Galun, 1977). Plants heterozygous at this locus (Ss) have the "thrum" flower type, and plants with the recessive genotype (ss) have the "pin" flower. A similar pleiotropic effect in P. fruticosa may be responsible for the conversion of some putative stamen primordia to petals under certain conditions.

Sawhney (1983) found GA to increase the number of all floral organs in Lycopersicon esculentum. If the recessive alleles at the D loci resulted in the reduced production or lack of a GA inhibitor, the total number of primordia on the androecial ridge would thus be expected to increase. This pleiotropic effect would account for the slopes of the regression lines of stamen number on petal number indicating slightly less than a 1:1 relationship.

In terms of the ongoing improvement program involving P. fruticosa at this institution, the genetic limit of 15 petals suspected by Robertson (1984) and Davidson (1986) appears to be illusory. At least one, and possibly more, additional recessive modifier genes have been found in this study, and by interbreeding progeny from the backcross population it may be possible to concentrate more recessive modifier genes. P. fruticosa 'Yellowbird', P. fruticosa 'Snowbird', and possibly P. fruticosa 'Orange Whisper' appear to have additional modifier genes which can increase petal number. Backcross progeny plants with over 22 petals should be

crossed to all three of these cultivars to determine the number and inheritance of these additional modifiers. A recent accession of P. f. 'McKay's White', a cultivar from Wisconsin with six to eight petals, could be used in future breeding programs as a possible source of novel genetic material. Interpollination of the irradiated population may also uncover recessive mutations which may also be of use in future breeding programs.

Phenotypic plasticity is a feature of P. fruticosa which complicates the genetic analysis of segregating populations because of its capacity to mask the expression of the genotype of the plants. Environmental influences may affect petal number (Robertson 1984), and therefore the ornamental value of a cultivar. In addition to facilitating genetic studies, stability of petal number is a major breeding objective. The developmental plasticity of this character should be thoroughly investigated to determine the existence of any selectable genetic variability in the stability of this and other characters. In vivo and in vitro studies of factors affecting plant hormone metabolism in the floral apex could be of use in this area.

The self-incompatibility system of P. fruticosa is a monogenic gametophytic system. The extent of the expression of the incompatibility reaction varies slightly between genotypes. For example, P. f. 'Grandiflora' and P. f. 'Maaneleyii' exhibit absolute self-incompatibility (Davidson

1986), but P. f. 'Snowbird' may occasionally set selfed seed, although the frequency of such self pollination is very low. The self-incompatibility of this species could be used to advantage in any large scale breeding program if the parents to be crossed were interplanted in an isolation block. Since bees are the most common pollinator, the most practical method of isolation would be in the form of enclosures rather than locating the plots at a distance from other plantings, as bees have a long foraging range and contamination from other plantings of P. fruticosa would be practically unavoidable.

Chapter VI

SUMMARY AND CONCLUSIONS

On the basis of the research conducted, the following conclusions may be drawn:

6.1 ONTOGENY OF EXTRA PETALS

1. The inflorescence of P. fruticosa is an indeterminate cyme. Each branch of the cyme consists of a terminal flower and two foliage leaves, from the axils of which the next order of inflorescence branches will develop. Terminal inflorescences develop on all shoots after a period of vegetative growth. The duration of the period of vegetative growth is greatly influenced by the environment, but if conditions permit, new inflorescences are initiated continuously over the growing season and each develops indefinitely until limited by environmental factors. This results in the continuous flowering habit characteristic of this species.
2. Petals and stamens originate from the same zone on the floral apex, the androecial ridge. Petals and stamens appear to form a continuum on the androecial ridge in that initially primordia are indistinguisha-

ble, and certain primordia may differentiate into either petals or stamens depending on genetic and environmental factors. Quantitative analysis of petal and stamen numbers confirms that extra petals arise at the expense of stamens. Therefore, conceivably, mutants may be able to be isolated in which all of the primordia on the androecial ridge differentiate into petals, or conversely into staminoid structures.

6.2 INHERITANCE OF PETAL NUMBER

1. The production of extra petals in P. fruticosa is controlled by two major duplicate genes, D_1 and D_2, which are inherited independently. Production of approximately one whorl of five extra petals results when either of these genes is homozygous recessive.
2. The degree of doubleness is controlled by one major modifier gene, Dm_1, and at least one minor modifier gene, Dm_2. All of the genes affecting doubleness are recessive and appear to act sequentially, that is there is a definite hierarchy of gene action, and each gene is only expressed when all of the preceding genes are expressed. The hierarchical order of these genes is D_1 and D_2 (the duplicate genes) on the same level, Dm_1 on the next level, and the minor modifier gene(s) on the next level. All of these genes are

inherited independently, and act in an additive manner to control petal number.

3. The Dm_1 locus is closely linked to the self-incompatibility (S) locus. This has the effect of altering segregation ratios in a backcross program and may complicate any attempt at inbreeding to develop homozygous lines.

6.3 SELF-INCOMPATIBILITY

1. The mature pollen of P. fruticosa is binucleate, a condition typical of species with a homomorphic gametophytic self-incompatibility system.
2. The self-incompatibility system in P. fruticosa is controlled by a single multi-allelic gene (S) acting gametophytically in the pollen. P. f. 'Maaneleyi' and P. f. 'Grandiflora' have no S alleles in common. P. f. 'Snowbird' and P. f. 'Yellowbird' also have no S alleles in common.

6.4 IRRADIATION OF CUTTINGS

1. The LD-50 for rooted greenwood cuttings of P.fruticosa exposed to gamma radiation at a dose rate of 120 krad/hr. was determined to be approximately 5-6 krad.

Chapter VII

SUGGESTIONS FOR FURTHER RESEARCH

The following areas are in need of further study:

1. Environmental factors affecting the duration of the vegetative period prior to inflorescence initiation.
2. Nutritional and environmental factors affecting inflorescence initiation and development.
3. The number and nature of modifier genes controlling the highest petal number class.
4. Intensity of linkage of the S and Dm_1 loci. Plants recessive for both the D_1 and D_2 loci such as YB X SB #47 and SB X YB #79 would be suitable for this study.
5. Time of D gene action.
6. Further intercrossing of P. fruticosa 'Snowbird', P. fruticosa 'Yellowbird', and (SB X YB)#79 to determine the source of the altered segregation observed.
7. Hormonal factors affecting petal and stamen determination.
8. In vivo studies of hormone balances in vegetative and floral apices of single and double flowered plants.
9. In vitro studies of the effects of plant hormones on cultured floral apices of single and double flowered plants.

10. Severe pruning of irradiated material to force the development of adventitious buds.
11. Interpollination or the collection of open-pollinated seed from irradiated material to uncover recessive mutants.
12. Incorporation of P. f. 'McKay's White' into a crossing program as a possible source of new genes for extra petals.
13. Examination of overwintering buds to determine whether any floral buds overwinter or are formed only on the current year's growth.

Chapter VIII

REFERENCES CITED

Abo El-Nil, M.M. and Hildebrandt, A.C. 1973. Origin of androgenetic callus and haploid geranium plants. Can. J. Bot. 51:2107-2109.

Asker, S. 1970. An intergeneric Fragaria X Potentilla hybrid. Hereditas 64:135-139.

Baer, I. and Kho, Y.O. 1971. An investigation into the cause of sterility in double flowered Freesia varieties and the possibility of restoring fertility. Euphytica 20:493-497.

Bateson, W. and Sutton, I. 1919. Double flowers and sex linkage in Begonia. J. Genet. 8:199-207.

Bauer, R. 1957. The induction of vegetative mutations in Ribes nigrum. Hereditas 43:323-327.

Beatty, A. 1937. A statistical study of flower doubling. Genetica 19:447-494.

Bowen, H.J.M., Cawse, P.A., Dick, M.J. 1962. The induction of sports in chrysanthemums by gamma radiation. Radiation Botany 1:297-303.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13:115-155.

Brewbaker, J.L. 1957. Pollen cytology and self-incompatibility systems in plants. J. Hered. 48:271-277.

Brewbaker, J.L.. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Am. J. Bot. 54:1069-1083.

Briggs, R.W. 1970. Radiation types and sources. In Manual on mutation breeding. IAEA/FAO. Vienna. pp.9-20.

Broertjes, C. 1972. Mutation breeding of Achimenes. Euphytica 21:48-62.

Broertjes, C. 1976. Mutation breeding of autotetraploid Achimenes cultivars. Euphytica 25:297-304.

Broertjes, C., Koene, P., Van Veen, J. W. H. 1980. A mutant of a mutant of a mutant of a ... : Irradiation of progressive radiation-induced mutants in a mutation breeding program with Chrysanthemum morifolium Ram. Euphytica 29:525-530.

Cheung, M. and Sattler, R. 1967. Early floral development of Lythrum salicaria. Can. J. Bot. 45:1609-1618.

Clark, G. 1981. Staining Procedures. (4th. Ed.) Williams and Wilkins. Baltimore. 512 pp.

Crane, M.B. and Lawrence, W.J.C. 1938. The Genetics of Garden Plants. MacMillan and Co. Ltd. London. 301 pp.

Davidson, C.G. 1986. Experimental taxonomy, propagation and models of inheritance for flower color and extra petals in Potentilla fruticosa L. Ph.D. Thesis. University of Manitoba. 320pp

Emino, E.R. 1966. Shoot apex development in the carnation (Dianthus caryophyllus). Proc. Am. Soc. Hor. Sci. 89:615-619.

Emino, E.R. and Rasmussen, H.P. 1971. Scanning electron microscope studies of the shoot apex in Dianthus caryophlyllus L. cv. Sania. J. Am. Soc. Hort. Sci. 96:253-256.

Ewart, L. 1984. Plant Breeding. In Petunia (K. Sink Ed.) Springer Verlag. Berlin. pp.180-202.

Ferran, T.E. and Wallace, D.H. 1977. Incompatibility of Brassica stigmas overcome by treating pollen with cycloheximide. Science 196:436-438.

Fourrier, B. 1985. Potentilla 'McKay's White' (Hybrid). AABGA Bulletin. July 1985. p.91.

Frankel, R. and Galun, E. 1977. Pollination Mechanisms, Reproduction and Plant Breeding. Springer Verlag. Berlin. 281 pp.

Garrod, J.F. and Harris, G.P. 1974. Studies on the glasshouse carnation: Effects of temperature and growth substances on petal number. Ann. Bot. 38:1025-1031.

Heslop-Harrison, J. 1956. Auxin and sexuality in Cannabis sativa. Physiol. Plant. 9:588-597.

Hicks, G.S. 1973. Studies on tobacco petal primordia in culture: Petal duplication induced by surgery. Bot. Gaz. 134:154-160.

Horridge, J.S. and Cockshull, K.E. 1974. Flower initiation and development in the glasshouse rose. Scientia. Hort. 2:273-284.

Jackson, G. 1934. The morphology of the flowers of Rosa and certain closely related genera. Am. J. Bot. 21:453-466.

Jahn, O.L. and Dana, M.N. 1970. Crown and inflorescence development in the strawberry, Fragaria ananassa. Am. J. Bot. 57:605-612.

Jelenkovic, G., Wilson, M.L., Harding, P.J. 1984. An evaluation of intergeneric hybridization of Fragaria spp. X Potentilla spp. as a means of haploid production. Euphytica 33:143-152.

Johansen, D.A. 1940. Plant Microtechnique. McGraw Hill, N.Y. 491p.

Kindiger, B. and Beckett, J.B. 1985. A hematoxylin staining procedure for maize pollen grain chromosomes. Stain Tech. 60:265-269.

Konzak, C.F., Conger, B.V., Nilan, R.A. 1970. Radiation sensitivity and modifying factors. In Manual on mutation breeding. IAEA/FAO Vienna. pp 44-47.

Lammerts, W.E. 1945. The breeding of ornamental edible peaches for mild climates. I. Inheritance of tree and flower characters. Am. J. Bot. 32: 53-61.

Lapins, K. 1962. Note on compact mutants of Lambert cherry produced by ionoizing radiation. Can. J. Pl. Sci. 43:424-425.

Lapins, K. 1965. Compact mutants of apple induced by ionizing radiation. Can. J. Pl. Sci. 45:117-124.

Lindenbaum, S., Ginzberg, C., Halevy, A.H. 1975. A morphological study of the bullhead malformation in the Baccara rose. Ann. Bot. 39:219-223.

Lundqvist, A. 1954. Studies on self sterility in rye, Secale cereale L. Hereditas 40:278-294.

MacFarlane Smith, W.H. and Jones, J.K. 1985. Intergeneric crosses with Fragaria and Potentilla. I. Crosses between Fragaria moschata and Potentilla fruticosa. Euphytica 34:725-735.

MacKay, G.R. 1977. The introgression of S alleles into forage rape, Brassica napus L. from turnip, Brassica campestris L. ssp. rapifera. Euphytica 26:511-519.

Meyer, V.G. 1966. Flower abnormalities. Bot. Rev. 32:165-195.

McIntosh, D.L. and Lapins, K. 1966. Differences in susceptibility to apple powdery mildew observed in McIntosh clones after exposure to ionizing radiation. Can. J. Pl. Sci. 46:619-623.

Micke, A. and Donini, B. 1982. Use of induced mutations in improvement of seed propagated crops. In Induced Variability in Plant Breeding. (C. Broertjes Ed.) Center for Agricultural Publishing and Documentation. Wageningen. pp.2-9.

Moe, R. 1971. Factors affecting flower abortion and malformation in roses. Physiol. Plant. 24:291-300.

Natarella, N.J. and Sink, K.C. 1971. The morphogenesis of double flowering in Petunia hybrida Hort. J. Am. Soc. Hort. Sci. 96:600-602.

North, C. 1979. Plant Breeding and Genetics in Horticulture. The MacMillan Press Ltd. London. 150 pp.

Nougarede, A. 1967. Experimental cytology of the shoot apical cells during vegetative growth and flowering. Int. Rev. Cytol. 21:203-351.

Nougarede, A., Gifford, E.M.J., and Rondet, P. 1965. Cyto-histological studies of the apical meristem of Amaranthus retroflexus under varying photoperiodic regimes. Bot. Gaz. 126:248-298.

Nugent, P.E. and Snyder, R.J. 1967. The inheritance of floret doubleness, floret center color, and plant habit in Pelargonium hortorum Bailey. Proc. Am. Soc. Hort. Sci. 91:680-690.

Nybom, N. 1970. Mutation breeding of vegetatively propagated plants. In Manual on mutation breeding. IAEA/FAO. Vienna. pp.141-149.

Ockendon, D.J. 1978. Effect of hexane and humidity on self-incompatibility in Brassica oleraceae. Theoret. Appl. Genet. 52:113-117.

Ordnuff, R. 1980. The probable genetics of distyly in Gelsemium sempervirens (Loganiaceae). Can. J. Genet. Cytol. 22: 303-304.

Osterbye, U. 1975. Self-incompatibility in Ranunculus acris L. I. Genetic interpretation and evolutionary aspects. Hereditas 80:91-112.

Pandey, K.K. 1977. Mentor pollen: possible role of wall held pollen growth promoting substances in overcoming inter and intraspecific incompatibility. Genetics 47:219-230.

Pearson, O.H. 1983. Heterosis in vegetable crops. In Heterosis, Reappraisal of Theory and Practice. (R. Frankel Ed.) Springer Verlag. Berlin. pp.138-188.

Pike, L.M. and Peterson, C.E. 1969. Gibberellin A4/A7 for induction of staminate flowers from the gynoecious cucumber (Cucumis sativus L.). Euphytica 18:101-105.

Raman, K. and Greyson, R.I. 1977. Changes during development in the compartmentalization patterns of extractable gibberellin-like substances in "single" and "double" genotypes of Nigella damascena. Can. J. Bot. 55:2115-2121.

Raman, K. and Greyson, R.I. 1978. Further observations on the differential sensitivities to plant growth regulators by cultured "single" and "double" flower buds of Nigella damascena L. (Ranunculaceae). Am. J. Bot. 65:180-191.

Reimann-Philipp, R. 1983. Heterosis in ornamentals. In Heterosis, Reappraisal of Theory and Practise. R. Frankel Ed. Springer Verlag. Berlin. pp.234-259.

Remphrey, W.R. and Steeves, T.A. 1984. Shoot ontogeny in Arctostaphylos uva-ursi (bearberry): Origin and early development of lateral vegetative and floral buds. Can. J. Bot. 62:1933-1939.

Reynolds, J. and Tampion, J. 1983. Double Flowers, a Scientific Study. Van Nostrand Reinhold Co. Inc. N.Y. 183 pp.

Rhodes, H.L.J. 1954. The cultivated shrubby Potentilla. Baileya 2:89-96.

Robertson, M. 1984. The effect of environmental factors on flower color and petal number of Potentilla fruticosa L. M.Sc. Thesis. University of Manitoba. 118pp.

Robinson, R.W., Whitaker, T.W., and Bohn, G.W. 1970. Promotion of pistillate flowering in Cucurbita by 2-chloroethylphosphonic acid. Euphytica 19:180-183.

Roggen, H. P. and Van Dijk, A. J. 1976. Thermally aided pollination: A new method of breaking self-incompatibility in Brassica oleracea L. Euphytica 25:643-646.

Roggen, H. P., Van Dijk, A. J., and Dorsman, C. 1972. Electric aided pollination: A method of breaking incompatibility in Brassica oleracea L. Euphytica 21:181-184. Ronald, W.G. and Ascher, P.D. 1975. Effects of high temperature treatments on seed yield and self-incompatibility in Chrysanthemum. Euphytica 24:317-322.

Rousi, A. 1968. Cytoplasmic inheritance in Aquilegia vulgaris. Hereditas 60:223-232.

Salisbury, F.B. and Ross, C.W. 1985. Plant Physiology. Wadsworth Publishing Company. Belmont Ca. 540 pp.

Sampson, D.R. and Cameron, D.F. 1965. Inheritance of bronze foliage, extra petals and pendulous habit in ornamental crabapples. Proc. Am. Soc. Hort. Sci. 86:717-722.

Sattler, R. 1973. Organogenesis of Flowers. University of Toronto Press. Toronto. 207 pp.

Sawhney, V.K. 1983. The role of temperature and it's relationship with gibberellic acid in the development of floral organs of tomato (Lyopersicon esculentum). Can. J. Bot. 61:1258-1265.

Sawhney, V.K. and Greyson, R.I. 1972. On the initiation of the inflorescence and floral organs in tomato (Lycopersicon esculentum). Can. J. Bot. 50:1493-1495.

Sawhney, V.K. and Greyson, R.I. 1979. Interpretations of determination and canalization of stamen development in a tomato mutant. Can J. Bot. 57:2471-2477.

Sears, E.R. 1956. The transfer of leaf rust resistance from Aegilops umbellulata to wheat. Brook. Symp. Biol. Genet. 9:1-22.

Sinnott, E.W. 1960. Plant Morphogenesis. McGraw Hill. N.Y.

Sparrow, A.H., Rogers, A.F., Schwemmer, S.S. 1968. Radiosensitivity studies with woody plants. I. Acute gamma irradiation survival data for 28 species and predictions for 190 species. Radiation Botany 8:149-186.

Strickberger, M.W. 1985. Genetics. MacMillan Publishing Co. N.Y. 842 pp.

Van Harten, A.M. 1982. Mutation breeding in vegetatively propagated crops with emphasis on contributions from the Netherlands. In Induced Variability in Plant Breeding. (C. Broertjes Ed.) Center for Agricultural Publishing and Documentation. Wageningen. pp.22-31.

Wetzstein, H.Y. and Armitage, A. 1983. Inflorescence and floral development in Pelargonium X hortorum. J. Am. Soc. Hort. Sci. 108:595-600.

Wilson, F.D. 1987. Inheritance of pink filament in cotton. J. Hered. 78:223-224.

Appendix A

CODE KEY TO NUMBERED CROSSES AND TREATMENTS

Crosses

1.	YB X (YB X OW)#4	5.	OW X (YB X OW)#4
2.	YB X (YB X OW)#5	6.	OW X (YB X OW)#5
3.	YB X (YB X OW)#44	7.	OW X (YB X OW)#44
4.	YB X (YB X OW)#112	8.	OW X (YB X OW)#112
9.	YB X (YB X PW)#19	13.	PW X (YB X PW)#19
10.	YB X (YB X PW)#30	14.	PW X (YB X PW)#30
11.	YB X (YB X PW)#93	15.	PW X (YB X PW)#93
12.	YB X (PW X YB)#100	16.	PW X (PW X YB)#100
17.	YB X (YB X SB)#15	21.	SB X (YB X SB)#15
18.	YB X (YB X SB)#46	22.	SB X (YB X SB)#46
19.	YB X (YB X SB)#47	23.	SB X (YB X SB)#47
20.	YB X (YB X SB)#50	24.	SB X (YB X SB)#50
25.	YB X (SB X YB)#21	28.	SB X (SB X YB)#21
26.	YB X (SB X YB)#32	29.	SB X (SB X YB)#32
27.	YB X (SB X YB)#79	30.	SB X (SB X YB)#79
31.	(YB X SB)#15 X YB	32.	(YB X SB)#15 X SB
33.	(YB X SB)#46 X YB	34.	(YB X SB)#46 X SB
35.	(YB X SB)#47 X YB	36.	(YB X SB)#47 X SB
37.	(YB X SB)#50 X YB	38.	(YB X SB)#50 X SB

39. (SB X YB)#21 X YB
40. (SB X YB)#21 X SB
41. (SB X YB)#32 X YB
42. (SB X YB)#32 X SB
43. (SB X YB)#79 X YB
44. (SB X YB)#79 X SB
45. SB X YB
46. YB X SB

Treatments

47. PW, 5krad
48. PW, 4krad
49. PW, 6krad
50. PW, control
51. OW, 6krad
52. OW, 5krad
53. OW, 4krad
54. OW, control
55. YB, 6krad
56. YB, 5krad
57. YB, 4krad
58. YB, control
59. SB, 6krad
60. SB, 5krad
61. SB, 4krad
62. SB, control
63. SB, 8krad
64. SB, 2krad
65. SB, 3krad
66. SB, 10krad
67. SB X SB

Appendix B

NUMBERS OF SEED AND PROGENY OBTAINED FROM BACKCROSS PROGRAM

Cross	Seed	Progeny	Cross	Seed	Progeny
1	116	42	23	622	95
2	118	58	24	707	134
3	483	177	25	566	234
4	164	58	26	144	25
5	122	18	27	450	93
6	90	9	28	678	46
7	73	8	29	450	102
8	23	1	30	650	67
9	30	0	31	332	56
10	182	78	32	659	32
11	248	46	33	202	41
12	9	0	34	400	103
13	1	0	35	163	23
14	66	11	36	251	56
15	87	16	37	373	84
16	39	10	38	500	67
17	168	24	39	500	68
18	205	64	40	500	52
19	211	91	41	4	0
20	204	54	42	2	0
21	398	55	43	500	72
22	224	44	44	500	58

Appendix C

Field Planting Plan by Cross or Treatment Number

Numbers in parentheses refer to numbers of plants in incomplete plots

Plot \ Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
25	37	22	29	43	24	45	36	27	37	23	27	25	45	21	39	32	24	40	45	46	27	19	46	32	37											
24	3	34	24	30	31	27	45	11	32	25	24	46	20	46	35	4	25	11	24	43	45	36	32	24	10											
23	4	27	2	18	45	44	34	3	24	34	19	34	10	31	46	5	38	24	46	37	28	28	1	18	4	3										
22	40	1	10	33	3 58(1)	19	24	45	43	23	23	25	3	26	11	36	19	45	45	45	44	25	36	31	37	29	31									
21	20	10	24	45	29(3)	3	46	25	46	25	45	36	20	40	2	30	30	26	39	3	45	5	10	46	45	36	10	23								
20	3	25	45	34	46	27	39	36	21	17	3	43	38	46	7	17	45	29	22	44	3	40	45	29	43	24	19	46	5	37						
19	40	2	24	20	34	10	20	43	34	34	15	25	27	25 11(2)	46	18	46	45	50(2) 28(2)	31	21	46	22	35	27	25	31	3	4	47						
18	36	30	26	24	24	3	29	25 62(1)	45	23	44	33	46	33(1) 54(1)	45	29	19	22	27	45	31	2	23	43	4	37	30	14	27	54	49	40				
17	37	2	44	45	6	23 50(1)	39	11(3)	31 6(1)	10	46	29	18	18	22	45	58(2) 24(2)	37	3	3	54(2) 44(2)	45	3	25	3 50(2)	45	46	34	21	61	56	49	58			
16	24 58(1)	46	3	38	40	30(3)	34	38	4(2) 58(1)	45	2	40	25	3	25	22	30	25	25	46	46	37	18	32	54(2)	27	45	45	32	56	65	55	54	56		
15	34(3)	25	15	1	4	18	25	45	21	25	45	25	46 58(1)	45	38	24	31	24	28	23	34	43	19	11	11	30	28	37	35	54	60	58	62	59	61(1) 58(1)	
14	22	45	45	4 62(1)	39	24	27	3	25	11	3	29	27(1) 5(2)	20	46	40	24	25	11	29	19	3 54(1)	39	18	28	24	39	24	23	65	50	59	55	52	63(3) 61(2)	
13	25	46	39 50(1)	21(3)	45	16	25	39	29	30	10	25	25	38	24	39	27	45	34	34	46	45(3)	2	45	3	10	25	45	46	58	56	50	53	48	52(3) 54(1)	39
12	39	3	19(3)	44	37	24	19	46	14	25 62(1)	29	45 62(1)	45	19	23	3	45	46	25	46	39	45	10	25	45	21	31	54	24	38	58	49	55	59	60(1) 61(2)	30
11	19	45 54(1)	4	25	17	20	34	1	6	20(3)	45	26(1) 2(2)	22	2	2	37	23	3	10	34	3	34	3	34	27	4	46	40	26	19	50	5	57	48	53(2) 57(3)	39
10	30	14(3)	29	44	26	34	38 62(1)	46	46	24	15	1	10	5	10 46(1)	36	37	19	38	25	46	36	45	33	18	23	17	30	25	31	49	52	62	55	55(2) 47(2)	43
9	19	2	18	38	46	27	3(1) 1(2)	3	18	37	34	16	25	38	50(1) 16(2)	38	25	33	25	62(2) 29(2)	38	1	32	3	19	21	10	28	25	3	57	59	54	52	61	44
8	22	33	46	44	35	45	43	17	23	30	21	46	23	25	27	19	23	45	4	46	3	45	25	23	21	3	35	45	23	15	61	61	62	60	60	23
7	39	29	31	39	29	43	23	45	45	4	40	25	3	46	37	45	3	54(2) 25(2)	3	31	46	37	27	46	46	36	18	25	38	23	57	58	59	47	55	28
6	1	27	29	19	44	22	32	34	3	25	45	22	28	33	29	62(2) 20(2)	29	22	27	44	34	23	45	32	34	3	3	23	39	34	61	64	52	52	62	44
5	28	29	45	3	19	45	3	30	33	23	25 50	20	23	1	19	27	30	23	25	18	43	2	25	1	39	18	18	28	11	23	50	61	66	60	53	17(4) 48(4) 51(1)
4	46	27	36	45	39	46	37	31	4	46	8 10	23	46	25	3	37	4	25	24	30	45	7	19	36	29	25	43	25	23	31	52	58	51	47	62	53(1) 57(2)
3	45	21	19	35	45	2	1	10	29	43	18	23	45	45	24	22	29	20	20	45	27	20	33	10	3	24	40	58	21	44	50	47	57	51	54	52(1) 48(2)
2	28	19	28	45	36	24	24	25	4	21	45	29	2	22	45	27	21	38	33 44(3)	25	30	38	10	45	43	27	3	10	36	34	60	53	54	58	62	65(1) 59(1)
1	11	45	25	24	43	34	29	24	37	45	38	45	25	3	25	45	46	25	67(1)	38	37	34	2	24	23	39	25	33	23	11	51	57	61	56	60	64(2) 60(1)

Appendix D

STAINING SCHEDULE FOR SECTIONS

Slides were immersed for 15 min. in:

1. 100% Xylene
2. 100% Xylene

5 min. in:

3. Equal parts 100% Xylene and 100% Ethanol
4. 100% Ethanol
5. 95% Ethanol
6. 80% Ethanol
7. 70% Ethanol
8. 50% Ethanol
9. Safranin O (1% in 50% Ethanol

The slides were rinsed in two quick changes of distilled water, and then immersed for 1 min. in:

10. 50% Ethanol
11. 70% Ethanol
12. 80% Ethanol
13. 95% Ethanol

2 min. in:

14. Fast Green FCF (0.5% in 95% 95% Ethanol)

1 min. in:

15. 95% Ethanol
16. 95% Ethanol
17. 100% Ethanol

2 min. in:

18. Equal parts 100% Xylene and 100% Ethanol

The slides were then transferred to 100% Xylene and held for at least 10 min. or until ready for mounting. Number 1 cover slips were mounted with Permount.

Appendix E

DEHYDRATION SCHEDULE FOR SEM SAMPLES

The following operations were conducted at 4oC. After washing out the fixatives with 0.2M Na-Phosphate buffer (pH=6.8), the samples were immersed for 1 h. in:

1. 50% Ethanol
2. 70% Ethanol
3. 80% Ethanol
4. 90% Ethanol
5. 95% Ethanol
6. 100% Ethanol

15 min. in:

7. 100% Ethanol
8. 100% Ethanol

The samples were then stored overnight in the freezer (-15oC.) in another change of 100% Ethanol. All subsequent operations were carried out at -15oC.

9. Two changes (15 min. each) of 100% Ethanol
10. One hour in Ethanol/Acetone (equal parts)
11. At least four changes (15 min. each) of 100% Acetone.

Appendix F

SURVIVAL OF IRRADIATED CUTTINGS

'Pink Whisper'	0 krad	96%	'Yellowbird'	0 krad	100%
	4 krad	56%		4 krad	68%
	5 krad	56%		5 krad	80%
	6 krad	64%		6 krad	56%
'Orange Whisper'	0 krad	84%	'Snowbird'	0 krad	88%
	4 krad	44%		4 krad	68%
	5 krad	24%		5 krad	74%
	6 krad	36%		6 krad	49%