

**Reproductive Biology of the Perennial Desert Shrub,  
*Thymelaea hirsuta*: Studies in Gender,  
Self-fertility and Architecture**

**by  
Rita Morbia**

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## ABSTRACT

The modular construction of plants allows them to display significant phenotypic plasticity in both morphology and sex expression. *Thymelaea hirsuta* (Thymelaeaceae) is a xerophytic evergreen shrub common to the Mediterranean region and known to demonstrate significant variability in sex expression. A series of observational and experimental studies were carried out to investigate the nature of this variability in greenhouse-raised plants. Seeds were obtained from Egyptian populations, and following seedling establishment, an irrigation treatment was applied. Plants were watered every 3 days, 6 days or 9 days.

A survey of two year old flowering plants ( $n = 305$ ) showed significant bimodality in sex expression. Quantitative gender estimates indicated that individual plants tended to be either more male or more female. Water treatment had no significant effect upon estimates of gender, number of male flowers, number of female flowers, total number of flowers or an index of sexual lability. A controlled pollination study revealed significant differences in seed mass among geitonogamous, half-sib and outcrossed treatments, resulting in an estimate of inbreeding depression ( $\delta$ ) of 0.23. Nonsignificant differences in fruit set were also observed. In general, selfing resulted in less fit progeny.

To examine the effect of water treatment on various morphological ("architectural") parameters, repeated measurements were taken every two months on a subset of 202 plants for a total of ten months. Water treatment, time and the interaction effect between water treatment and time all significantly affected plant architecture, particularly height. Male, female and hermaphrodite flowers were compared for differences in position. There were significant differences among male, female and hermaphrodite flowers with respect to plant height, vertical distribution and branch order, suggesting that male flowers and female flowers may have different

**morphological optima.**

**These studies indicate that selection for the evolution of dioecy in *Thymelaea hirsuta* may involve a combination of selection for outcrossing and resource or pollinator efficiency.**

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## TABLE OF CONTENTS

|   |      |
|---|------|
| ABSTRACT . . . . .  | iii  |
| ACKNOWLEDGEMENTS . . . . .                                    | v    |
| LIST OF TABLES . . . . .                                      | viii |
| LIST OF FIGURES . . . . .                                     | xi   |
| CHAPTER   |      |
| <br>  |      |
| 1. GENERAL INTRODUCTION                                       |      |
| Plant Sex Expression . . . . .                                | 1    |
| Gender Quantification . . . . .                               | 10   |
| Mating Systems . . . . .                                      | 13   |
| Maternal and Paternal Influences on Seed Production . . . . . | 17   |
| Secondary Sex Characteristics . . . . .                       | 25   |
| Evolution of Dioecy . . . . .                                 | 30   |
| Plant Architecture . . . . .                                  | 40   |
| <i>Thymelaea hirsuta</i>                                      |      |
| i) General Description . . . . .                              | 47   |
| ii) Sex Expression . . . . .                                  | 54   |
| iii) Evolution of Dioecy in the Thymelaeaceae . . . . .       | 61   |
| <br>  |      |
| 2. GENDER STUDY   |      |
| Objective . . . . .   | 64   |
| Materials and Methods . . . . .                               | 66   |
| Results . . . . .   | 72   |
| Discussion . . . . .  | 98   |

|  |            |
|--|------------|
| <b>3. SELF-FERTILITY STUDY</b>         |            |
| <b>Objective</b> . . . . .             | <b>109</b> |
| <b>Materials and Methods</b> . . . . . | <b>110</b> |
| <b>Results</b> . . . . .               | <b>113</b> |
| <b>Discussion</b> . . . . .            | <b>129</b> |
| <br>                                   |            |
| <b>4. ARCHITECTURE STUDY</b>           |            |
| <b>Objective</b> . . . . .             | <b>134</b> |
| <b>Materials and Methods</b> . . . . . | <b>135</b> |
| <b>Results</b> . . . . .               | <b>138</b> |
| <b>Discussion</b> . . . . .            | <b>157</b> |
| <br>                                   |            |
| <b>GENERAL DISCUSSION</b> . . . . .    | <b>165</b> |
| <br>                                   |            |
| <b>LITERATURE CITED</b> . . . . .      | <b>170</b> |
| <br>                                   |            |
| <b>VITA AUCTORIS</b> . . . . .         | <b>183</b> |

## LIST OF TABLES

### Chapter 1

|  |    |
|--|----|
| Table 1.1 Summary of gender phenotypes reported for <i>Thymelaea hirsuta</i> . . . . . | 55 |
|--|----|

|   |    |
|---|----|
| Table 1.2. Description of gender phenotypes in <i>Thymelaea hirsuta</i> , according to El-Keblawy et al. (1995) . . . . . | 60 |
|---|----|

### Chapter 2

|   |    |
|---|----|
| Table 2.1. Mean gender value and variance of male and female "clusters" of <i>Thymelaea hirsuta</i> , using all flowering plants, and only intermediate gender values . . . . . | 76 |
|---|----|

|  |    |
|--|----|
| Table 2.2. Summary of gender phenotypes for two year old plants of <i>Thymelaea hirsuta</i> , raised under greenhouse conditions . . . | 77 |
|--|----|

|   |    |
|---|----|
| Table 2.3. Results of MANOVA for total number of flowers produced, total number of female flowers produced, total number of male flowers produced, gender value, sex lability index, days to first flowering and length of flowering period . . . . . | 78 |
|---|----|

|   |    |
|---|----|
| Table 2.4. Mean ( $\pm$ SE) number of days to first flowering and the effects of water treatment and site in <i>Thymelaea hirsuta</i> . . . . . | 79 |
|---|----|

|   |            |
|---|------------|
| <b>Table 2.5.a) Results of Chi-Square analyses to determine if proportions of male, female dichogamous and labile phenotypes differ significantly for field (from El-Keblawy et al. 1995) versus greenhouse populations . . . . .</b> | <b>80</b>  |
| <b>Table 2.5.b) Results of Chi-Square analyses to determine if proportions of male, female dichogamous and labile phenotypes differ significantly for field (from Ramadan et al. 1994) versus greenhouse populations . . . . .</b>    | <b>81</b>  |
| <b>Table 2.6. Results of maximum likelihood ANOVA from multiway frequency analysis testing associations among water treatment, maternal gender and water treatment . . . . .</b>  | <b>82</b>  |
| <br><b>Chapter 3</b>  |            |
| <b>Table 3.1. Number of pollinations of each cross type performed on flowering plants of <i>Thymelaea hirsuta</i> . . . . .</b>   | <b>117</b> |
| <b>Table 3.2. Results of ANOVA for effects of pollination treatment on proportion fruit set (plant as replication unit) . . . . .</b>   | <b>119</b> |
| <b>Table 3.3. Results of ANOVA for effects of pollination treatment on seed mass (plant as replication unit) . . . . .</b>  | <b>120</b> |
| <b>Table 3.4. Results of ANOVA for effects of pollination treatment on fruit set (flower as replication unit) . . . . .</b>   | <b>121</b> |
| <b>Table 3.5. Results of ANOVA for effects of pollination treatment on seed mass (flower as replication unit) . . . . .</b>   | <b>122</b> |

## **Chapter 4**

**Table 4.1. Results of repeated measures MANOVA testing the effects of water treatment and cohort (time) on height, primary length, secondary length, tertiary length, number of secondary branches and number of primary branches . . . . . 141**

**Table 4.2. Results of repeated measures MANOVA testing the effects of water treatment, and cohort (time) on height, total length of five primary branches (including all higher orders) and total number of branches on primary branches . . . . . 142**

## LIST OF FIGURES

### Chapter 1.

- Figure 1.1. Fitness (reproductive success) of either sex, as a function of resource allocation . . . . . 31
- Figure 1.2. Conditions for stability of an hermaphroditic or dioecious strategy where a concave curve defines the ESS for dioecy while a convex curve defines the ESS for hermaphroditism . . . . . 32
- Figure 1.3. Schematic drawing of a plant showing primary, secondary and tertiary branch orders . . . . . 42
- Figure 1.4. *Thymelaea hirsuta* . . . . . 48

### Chapter 2.

- Figure 2.1. Frequency distribution of plant gender values (indicating degree of femaleness) in *Thymelaea hirsuta* (n = 305). a) GV1 values are calculated by taking the mean of gender values at each cohort; b) GV2 estimates use the total number of male, female and hermaphrodite flowers produced by a plant to calculate mean gender.. . . . . 84
- Figure 2.2. Frequency distribution of plant gender values in *Thymelaea hirsuta* (n = 93), omitting pure male (gender value = 0.0) and pure female (gender value = 1.0) sex phenotypes . . . . . 85

**Figure 2.3. Mean gender value in *Thymelaea hirsuta* ( $\pm$  SE) for coastal dune sites (coast), depression sites (depression) and inland sites (inland) at irrigation treatment levels of 3 days, 6 days and 9 days . . . . . 87**

**Figure 2.4. Floral sex expression (number of female, male and hermaphrodite flowers) in *Thymelaea hirsuta* for coastal dune (coast), depression (depression) and inland (inland) sites at each watering treatment . . . . . 89**

**Figure 2.5. Frequency distribution of sex lability values in *Thymelaea hirsuta*, calculated by summing the difference between successive gender values determined over the course of the reproductive session (n = 305) . . . . . 92**

**Figure 2.6. Mean sex lability ( $\pm$  SE) values in *Thymelaea hirsuta* from coastal dune sites (coast), depression sites (depression) and inland sites (inland) for each water treatment . . . . . 93**

**Figure 2.7. Mean number of days ( $\pm$  SE) to first flowering for *Thymelaea hirsuta* plants from coastal dune, depression, inland sites for 3 day, 6 day and 9 day watering frequencies . . . . . 94**

**Figure 2.8. Comparison of frequencies of sexual phenotypes in greenhouse and field populations of *Thymelaea hirsuta* at coastal dune, depression, and inland sites . . . . . 95**

### **Chapter 3**

|   |            |
|---|------------|
| <b>Figure 3.1. Mean proportionate fruit set (<math>\pm</math>SE) per plant for each pollination treatment . . . . .</b>   | <b>124</b> |
| <b>Figure 3.2. Mean seed mass (<math>\pm</math>SE) per plant for each pollination treatment . . . . .</b>                 | <b>125</b> |
| <b>Figure 3.3. Mean proportionate fruit set (<math>\pm</math>SE) per flowers for each pollination treatment . . . . .</b> | <b>126</b> |
| <b>Figure 3.4. Mean seed mass (<math>\pm</math>SE) per flower for each pollination treatment . . . . .</b>                | <b>127</b> |
| <b>Figure 3.5. Mean proportionate seed germination (<math>\pm</math>SE) for each pollination treatment . . . . .</b>      | <b>128</b> |

### **Chapter 4.**

|   |            |
|---|------------|
| <b>Figure 4.1. Schematic drawing of <i>Thymelaea hirsuta</i>, showing how positional measurements were taken . . . . .</b>  | <b>145</b> |
| <b>Figure 4.2. Mean (<math>\pm</math>SE) plant height for each water treatment per cohort. Circles represent watering frequency of 3 days, squares represent watering frequency of 6 days and diamonds represent watering frequency of 9 days . . . . .</b> | <b>146</b> |

|  |     |
|--|-----|
| Figure 4.3. Mean ( $\pm$ SE) length of 5 primary branches for each water treatment. Circles represent watering frequency of 3 days, squares represent watering frequency of 6 days and diamonds represent watering frequency of 9 days . . . . . | 147 |
| Figure 4.4. Mean ( $\pm$ SE) secondary branch length for 5 primary branches demonstrating a significant effect of cohort . . . . .   | 148 |
| Figure 4.5. Mean ( $\pm$ SE) tertiary branch length for 5 primary branches per plant, showing a significant effect of cohort . . . . .   | 149 |
| Figure 4.6. Mean ( $\pm$ SE) number of secondary branches on 5 primary branches of <i>Thymelaea hirsuta</i> demonstrating a significant effect of cohort . . . . .   | 150 |
| Figure 4.7. Mean ( $\pm$ SE) number of tertiary branches on 5 primary branches of <i>Thymelaea hirsuta</i> demonstrating a significant effect of cohort . . . . .  | 151 |
| Figure 4.8. Mean ( $\pm$ SE) length of 5 primary branches and all higher order branches over all cohorts . . . . .   | 152 |
| Figure 4.9. Mean ( $\pm$ SE) number of branches of all orders on 5 primary branches in <i>Thymelaea hirsuta</i> . . . . .  | 153 |
| Figure 4.10. Mean ( $\pm$ SE) height for male, female and hermaphrodite flowers . . . . .  | 154 |
| Figure 4.11. Mean ( $\pm$ SE) branch order for male, female and hermaphrodite flowers . . . . .  | 155 |

**Figure 4.12. Mean ( $\pm$ SE) rank of primary branch measure for  
male, female and hermaphrodite flowers . . . . . 156**

# **Chapter 1**

## **General Introduction**

### **Plant Sex Expression**

Plant sexuality is a complex, variable phenomenon. As opposed to higher animals, that rarely depart from a gonochoristic gender strategy, higher plants may assume a wide array of sexual forms. Moreover, their sexuality may vary at a number of different levels, including the flower, the inflorescence, the individual plant and the population (Lloyd 1972, Richards 1986, Cox 1988, Barrett and Harder 1995). Any index measuring plant sexuality should include both spatial and temporal dimensions in order to adequately describe different types of variability.

Most plant species are hermaphroditic (Yampolsky and Yampolsky 1922), their flowers produce both male and female sex organs. The female sex organ or gynoecium includes the stigma (that facilitates pollen capture), the style (through which pollen tubes must grow) and the ovary. An ovary may contain one or more ovules. Fundamentally, it is the presence of ovules which confers femaleness in a flower. The male sex organ or androecium contains filaments, upon which rest the anthers, that form and disperse pollen grains. It is the ability to make pollen that confers maleness. Hermaphrodite flowers can potentially carry out both male and female function (but see Horovitz 1978).

### **Floral Variation in Sex Expression**

Hermaphrodite flowers can contribute to the next generation via contributions from both pollen and ovules. Angiosperm flowers are either monoecious, producing both ovule and pollen in a single bisexual

(hermaphrodite) flower or diclinous, where some flowers produce only ovules, while other flowers bear only pollen. (see Lloyd 1972, Richards 1986, Lovett Doust and Lovett Doust 1988, Cruden and Lloyd 1995). Thus, femaleness and maleness may be florally partitioned, resulting in unisexual as opposed to bisexual flowers.

Though the immediate regulation of floral gender is genetic, there remains a need to unify existing molecular and ecological information. Three different types of genes are involved in flower development: 1) organ identity genes whose functions most directly affect production of sepals, petals, stamens and carpels; 2) cadastral genes that determine the spatial expression of organ identity genes and; 3) meristem identity genes that are required to promote the activity of floral meristems (Weigel 1995, Weigel and Meyerowitz 1994). These genes are believed to be highly conserved.

Flower parts are simply modified leaves. Normal flowers consist of four concentric whorls arranged from outermost to innermost levels as sepals, petals, stamens and carpels, respectively (Weigel and Meyerowitz 1994). An understanding of organ identity gene functioning provides an explanation of the mechanism by which flower gender is governed. Each whorl can be converted into any other basic floral sex organ. Cadastral genes and meristem identity genes are both upstream regulators, however, the function of the former is poorly understood (Weigel and Meyerowitz 1994).

### **Spatial Variation in Plant Sexuality**

'Sex habit' refers to the spatial arrangement of sex organs (Lovett Doust and Lovett Doust 1988). Cruden and Lloyd (1995) use the term 'sexual phenotype' to describe this aspect of plant reproductive morphology while Richards (1986) simply refers to the "conditions with respect to the distribution of male and female organs" (pg. 285). More broadly, the terms 'sex expression' (Allison 1991) and 'gender variation' (Ågren and Willson

1991) have also been used to describe the spatial aspect of plant sexuality. A standard convention has yet to be agreed upon. However, I will use the term sex habit to refer explicitly to where male and female sex organs are located in space with respect to one another.

### **One Gender Class**

Through various combinations and permutations at the level of the flower, a large array of plant sex habits are possible. Most basically, we can consider plants where all individuals belong to the same gender class (or are 'homoecious') and contain only one flower type (Cruden and Lloyd 1995). In these plants all flowers are hermaphrodites and each flower has the ability to contribute to the next generation via ovule and pollen production.

The alternative to homoecious monoecy involves the production of a mixture of two or even three floral types per plant. Plants regularly containing both male and female flowers are called monoecious (Bawa and Beach 1981). If male and hermaphrodite flowers are present on all individuals, the sex habit is termed andromonoecy; analogously, gynomoecy describes the situation where there are female and hermaphrodite flowers on all plants. Another less common variant of a single gender class involves trimonoecy, the situation where male, female and hermaphrodite flowers are found on the same plant (Cruden and Lloyd 1995). Darwin (1877) referred to this condition as a "monoecious subgroup of polygamous plants" (pg. 12) while Yampolsky and Yampolsky (1922) called this sex habit simply 'polygamous'. It is also important to note that when there is more than one flower type present on a plant, variation in the relative proportion of male, female and hermaphrodite flowers may occur.

The majority of angiosperm species, (> 70%) are hermaphrodite in their sex habit (Yampolsky and Yampolsky 1922). A small subset of hermaphroditism is heterostyly where species consist of either two (distyly) or three (tristyly) flower morphs, differing in style length. There is a

relatively strong degree of incompatibility within morphs (Darwin 1877, Barrett 1988). Examples of distyly include members of the family Oleaceae such as *Jasminium fruticans* (Thompson and Dommée 1993). The families Lythraceae and Pontederiaceae contain examples of tristylous species such as *Lythrum salicaria* and *Eichhornia* spp., respectively.

An estimated 5% of all angiosperms are monoecious (Yampolsky and Yampolsky 1922). Examples include *Zea mays* (corn), where the androecia (or 'tassles') and gynoecia ('ears' and silks) are produced at the tip and base of the plant respectively. Other examples include *Typha* spp., *Quercus* spp., *Carex* spp. and nearly all species in the Pineaceae. Large, windpollinated species, especially trees and sedges, along with those utilizing hydrophilous pollination are largely monoecious (Richards 1986).

Andromonoecism and gynomonoecism are less frequent sex habits. Species containing both male and hermaphrodite flowers comprise only 1.7% of angiosperms (Yampolsky and Yampolsky 1922). Andromonoecy is found in *Solanum hirtum* (Diggle 1994) in the Solanaceae and many species of the Apiaceae such as *Smyrniolum olusatrum*, *Pastinaca sativa* and *Anthriscus sylvestris* (Lovett Doust 1980). Gynomonoecism is slightly more common, present in about 2.8% of angiosperm species (Yampolsky and Yampolsky 1922). Many members of the Asteraceae are gynomonoecious, including *Bellis* spp. and *Solidago* spp. (Silvertown and Lovett Doust 1993).

### **Two or More Gender Classes**

Some species consist of two or more gender classes in a population. Here, sex expression can vary at two levels: the flower as well as the plant. The most common spatial arrangements of sex organs in plant species with two gender classes are dioecy and gynodioecy. Dioecy describes the condition where unisexual male and female flowers are found on different

plants (e.g. see Beach and Bawa 1981). Only 4% of angiosperm species are dioecious (Yampolsky and Yampolsky 1922). Many willows (*Salix* spp.) are dioecious. Other examples include *Silene alba* in the Caryophyllaceae (Lovett Doust et al. 1987), *Rubus chamaemorus* (Korpelainen 1994) in the Rosaceae and several spp. of *Cotula*, including *C. serrulata*, *C. dioica*, and *C. squarida* in the Asteraceae (Lloyd 1972).

A larger proportion of angiosperms are gynodioecious, containing female morphs (which produce only female flowers) and hermaphrodite morphs (which produce only hermaphrodite flowers) (Darwin 1877). It is estimated that 7% of flowering plants demonstrate this sex habit (Yampolsky and Yampolsky 1922). Examples include *Scandia geniculata* in the Apiaceae (Webb 1992), *Silacea oregana* ssp. *spicata* (Ashman 1992) and *Thymus vulgaris* in the Lamiaceae (Assouad et al. 1978).

Androdioecy is an extremely rare sex habit. It is analogous to gynodioecy in that there are two sexual forms, but one kind of individual bears only male flowers and the other only hermaphrodite flowers. Even Darwin (1877) remarked on the paucity of this sex habit and the unlikelihood of it existing. However, a recent example of androdioecy has been reported in *Datisca glomerata* (Liston et al. 1990).

Trioecy (termed 'trioecious' by Darwin 1877) describes a population consisting of separate male, female and hermaphrodite individuals. Yampolsky and Yampolsky (1922) make no mention of a purely trioecious species, but Cruden and Lloyd (1995) refer to *Arisaema dracontium* as a possible example of trioecy. However, no hermaphrodite flowers of this species exist (R. Boles personal communication). Furthermore, there has only ever been one purely female individual documented (Clay 1993), providing little evidence that typically, pure female morphs exist in this species. *A. dracontium* consists of either male or monoecious individuals. However, a recent example of trioecy was reported in the cactus

*Pachycereus pringlei* (Fleming et al. 1994).

Subdioecy (Darwin 1877, Lloyd 1972), also known as polygamodioecy (Yampolsky and Yampolsky 1922, Primack and McCall 1986, Cox 1988, Cruden and Lloyd 1995), describes a dioecious situation where occasional flowers of the opposite sex are found on otherwise unisexual individuals. Lloyd (1972) also recognized the presence of occasional hermaphrodite flowers and was less stringent in his definition, stating only that subdioecious males and females are 'variable' and may produce gametes of both sexes (see also Yampolsky and Yampolsky 1922). A slightly different definition was given by Richards (1986) who partitioned subdioecy into two forms: subgynoecy, involving exclusively male individuals plus female individuals which also produced hermaphrodites; and subandroecy where there are exclusively female plants in the population as well as a individuals containing male and hermaphrodite flowers. Lloyd (1972) has described subdioecy in the genus *Cotula*, as has Webb (1979a) in *Gingidia montana*.

### **Temporal Variation in Sex Expression**

Another term for the spatial separation of sexual function either within flowers, or between flowers is herkogamy (Robertson and Lloyd 1991). Functional separation of maleness and femaleness can also be attained through temporal partitioning, a strategy termed dichogamy. There are two basic ways in which the nonsynchrony of stigmatic receptivity (female function) and pollen release (male function) may be achieved: protandrous or protogynous separation. Protandry involves pollen dehiscence before the occurrence of stigma receptivity. Protogyny is analogous but of the opposite sequence and stigma receptivity occurs prior to pollen release.

Some authors make no particular distinction between interfloral and intrafloral dichogamy (Cruden and Lloyd 1995, Richards 1986). However,

Bertin and Newman (1993) in their review state explicitly that "while the distinction between interfloral and intrafloral dichogamy has often been recognized, these levels have often been lumped in analyzing data. Our results show that such a treatment of data is inappropriate" (pg. 141). Dichogamy may be "complete", indicating that there is no overlap in male and female function or dichogamy may be "incomplete", indicating the presence of an intermediate transition period with simultaneous male and female reproductive activity (Bertin and Newman 1993). There are numerous examples of intrafloral protandry in the Asteraceae and Apiaceae, and of intrafloral protogyny in the Onagraceae and Apiaceae (Bertin and Newman 1993). Interfloral dichogamy appears to be less common. However, many examples of interfloral protandry exist in the Arecaceae and of interfloral protogyny in the Asteraceae and Araceae (Bertin and Newman 1993).

Heterodichogamy is a version of dichogamy where both protandry and protogyny are simultaneously displayed in a species. *Grayia brandegei* in the family Chenopodiaceae (Pendleton et al. 1988) is a good example of this phenomenon that has also been called temporal dioecism, synchronized dichogamy, second-order dichogamy (see Cruden and Lloyd 1995). The plant is monoecious but half of the individuals in the population demonstrate complete (interfloral) protogyny and the other half demonstrate complete (interfloral) protandry, synchronously (Pendleton et al. 1988).

### **Morphological (Phenotypic) vs. Functional Gender**

The present, largely typological nomenclature, describes where male, female or hermaphrodite sex organs are located with respect to one another and the individual plant. It is assumed that morphology reflects function, i.e., female flowers produce functional ovules, male flowers produce viable pollen grains and hermaphrodite flowers produce both. However, function is not necessarily determined by structure (Horovitz 1978). Flowers may have

nonfunctional sex organs, and the fitness of an individual plant via either maleness or femaleness may vary widely (Snow 1989).

Cryptic dioecy is a situation which exemplifies the potential dichotomy between reproductive form and function (Mayer and Charlesworth 1991). It is an extreme case of the inequality described by Horovitz (1978) where flowers that are anatomically both male and female, are in function, primarily unisexual.

Kawakubo (1990) examined three species in the genus *Callicarpa* (Verbenaceae) where flowers of *C. glabra*, *C. nisimurae* and *C. subpubescens* were all found to contain both a gynoeceium and an androeceium, except for occasional styleless flowers in *C. pubescens*. Morphologically, they appeared bisexual. However further examination revealed that there were two predominant morphs in these three species, a long-styled morph with nongerminating inaperturate pollen grains, and a short-styled (or styleless) morph that failed to set fruit (Kawakubo 1990). Consequently, these species were effectively dioecious.

Several species in the genus *Saurauia* exhibit androdioecy (Cane 1993). Some individuals bear solely hermaphrodite flowers, and others bear only staminate flowers (Cane 1993). Only pollen from male flowers is functional, another example of cryptic dioecy. Cane (1993) postulates that the foraging behaviour of bees explains the maintenance of expensive nonfunctional male parts in a functionally female flower. Male organs act as a nutritional attractant. Similarly, floral mimicry, under conditions when the female morph produces staminal hairs, to attract pollinators is observed in *Osyris alba* (Aronne et al. 1993). Nonfunctional sexual parts may be maintained by factors such as pollinator behaviour.

Differences in sexual contribution can also be explained in terms of the sex ratio. If hermaphrodites are equisexual, then each individual flower will have a male:female ratio of 1:1. However, "dissimilar selection pressures to which . . . male and female gametophytes are subjected make

this generality unlikely" (Horovitz 1978, pg. 485). Still, an equilibrium can be maintained as long as there is a 1:1 ratio at the level of the population (Horovitz 1978). If increases in male function occur concurrently with increases in female function or vice versa, the population sex ratio remains at equilibrium, allowing a discrepancy between gender morphology and function to exist.

## Quantitative Gender

It becomes apparent that variation in sex habit can produce enormous complexity as one attempts to fit more and more sexual conditions into the existing framework. Presently, there are two main problems which can arise. Firstly, the existing terminology does not describe all combinations of spatial sex organ arrangement. For example, *Taxus canadensis* consists of female individuals, male individuals and monoecious individuals (Allison 1991), a situation not described by existing sex habit terminology. Second, the presence of intermediate sexual phenotypes is problematic in the use of categorical nomenclature (Darwin 1877, Cox 1988).

Lloyd (1979) developed the notion of quantitative gender to circumvent these problems associated with defining sex habit purely categorically. He partitioned reproductive behaviour in terms of maternal and paternal success. Female (maternal) fitness was measured as the proportion of progeny resulting via ovule production ( $G_i$ ) and male fitness via pollen production success ( $1 - G_i$  or  $A_i$ ) (Lloyd 1979).

As well as progeny success, quantitative gender also includes an equivalence factor,  $E$ , the ratio of the sum of gynoecial units to the sum of androecial units (Lloyd 1979).  $E$  is necessary to standardize the "currency" employed to assess maleness and femaleness. An estimate of gynoecial production,  $g_i$ , such as ovary, fruit or seed number is required along with units of androecial production,  $a_i$ , such as the production of pollen or polleniferous flowers. Femaleness of an individual,  $G_i$ , is then defined as:

$$G_i = g_i / [g_i + a_i E]$$

and maleness,  $A_i$ , is defined in terms of femaleness as stated earlier (Lloyd 1979).

The use of Lloyd's gender index, also termed prospective gender (Lloyd 1980, Delesalle 1989), has been widespread. Delesalle (1989) used Lloyd's index to calculate a quantitative measure in monoecious *Apodanthera*

*undulata*. She also calculated morphological gender, a term estimated slightly differently by employing only androecial and gynoecial production without an equivalence factor. Femaleness was measured as the number of gynoecial units divided by the sum of androecial and gynoecial units ( $g_i / [g_i + a_i]$ ) and maleness was the proportion of androecial units ( $1 - g_i$ ). A feature of both morphological and prospective gender is that they vary continuously from 0, indicating a pure male form and 1, indicating a pure female form. A disadvantage to using prospective gender estimates involves the instability of the equivalence factor from population-to-population or year-to-year (Delesalle 1989). In fact, Delesalle (1989) found that morphological gender was more likely to remain consistent between years than prospective gender. Moreover, prospective gender estimates assume a 1:1 ratio in male:female reproductive investment (Lloyd 1979).

Klinkhamer et al. (1994) calculated prospective gender using seed production as the gynoecial parameter and polleniferous flower production as the androecial parameter. The femaleness value for *Echium vulgare*, a phenotypically gynodioecious species, was between 0.28 and 0.51 for hermaphrodites and 1.0 for female plants, by convention (Klinkhamer et al. 1994). In *Gurania spinulosa*, prospective gender also showed bimodality, with pure male vines ( $G_i = 0.0$ ) and female vines of gender  $> 0.60$  (Condon and Gilbert 1988). Allison (1992) used strobili counts as gender units in *Taxus canadensis*, and found that the prospective gender may be female-biased due to herbivory. Clay (1993) documented gender changes in *Arisaema dracontium* related to plant size using Lloyd's quantitative gender index.

However, alternate estimates of quantitative gender have also been developed. Ågren and Willson (1991) studied the gynodioecious *Geranium maculatum* and defined 'phenotypic gender' as the percentage of functional anthers. All plants yielded seed and so gender varied from pure females (0%

functional anthers) to hermaphrodite individuals (100% functional anthers). Approximately 20 - 27% of individuals demonstrated an intermediate gender phenotype with a partial proportion of functional anthers (Ågren and Willson 1991). El-Keblawy et al. (1995) determined quantitative gender estimates similar to Delesalle's (1989) morphological gender. The proportion of male, female and hermaphrodite flowers on a plant were estimated, and converted to a value indicating relative femaleness. This value varied continuously from 0.0, specifying a pure male plant to 1.0, specifying a pure female plant. The gender index was calculated by summing the proportion of female flowers and half the proportion of hermaphrodite flowers on a particular plant (El-Keblawy et al. 1995).

There are certain disadvantages to a quantitative gender index. Though intermediate values provide an overall estimate of gender, they do not convey information regarding temporal variation in gender, over the course of the reproductive period. Furthermore, the source of the gender value cannot be determined. For example, a morphological gender value of 0.5 would not tell us the nature of the gender strategy employed to produce this intermediate value. The plant may consist solely of hermaphrodite flowers, it may be monoecious, with equal proportions of male and female individuals, or it may be dichogamous and produce an equal number of male and female units, but only when measured at the end of the reproductive season. Moreover, the absolute magnitude of gender components is obscured. A gender value of 1.0 may indicate that the plant produced only one ovule over the course of its reproductive season, or it may have produced 5000 ovules. A combination of qualitative and quantitative observations more effectively and more completely assesses the nature of plant sex expression.

## **Mating Systems**

The mating system of a plant describes who mates with whom and has broad implications concerning the overall genetic structure of plant populations (Willson and Burley 1983, Silvertown and Lovett Doust 1993). Plants are very flexible in their reproductive abilities, and can produce progeny in a variety of ways, spanning a range from purely vegetative to fully sexual. Thus progeny may be complete clones of a parent or may have a widely different genotype. In sexual reproduction, the success of particular crosses is dependent on several factors.

Among the most important determinants of plant mating systems is the sex habit. The spatial arrangement of sex organs in a population has a large influence on whether matings will be within flowers, among flowers within a plant, or between different plants. Furthermore, the nature and extent of self-incompatibility will influence the success of pollinations that occur. It is important to distinguish between pollination and fertilization as pollination only refers to the transport of pollen onto a stigma. Fertilization is the fusion of two gametes and thus, in the context of plant reproduction, only reflects successful pollinations (Richards 1986).

Even among those who study pollination, there is little standardization of the terminology used. In the simplest case, pollen can be transferred from anther to stigma within a single hermaphrodite flower. This type of mating is called autogamy (Richards 1986). The potential for autogamy only exists in species having self-compatible hermaphrodite flowers. As well, the degree of intrafloral dichogamy may affect whether hermaphrodite flowers self-pollinate. Cruden and Lloyd (1995) have proposed that autogamy should reflect successful pollination and thus these terms should include the notion of fertilization.

Allogamous mating events occur among different flowers. If both staminate and pistillate flowers are found on the same plant, then there is a

potential for pollen transfer within a plant and self-fertilization can occur provided self-incompatibility systems are not present. This is called geitonogamy. Intrafloral dichogamy would be insufficient in preventing geitonogamous pollen transfer. In fact, dichogamy in general is a poor method of preventing selfing, unless complete interfloral separation exists (i.e. temporal dioecism) and there is no overlap in male and female function within a plant (Thomson and Brunet 1991). Sex habits including monoecy, gynodioecy and andromonoecy may facilitate transfer of pollen between flowers within plants since both male and female sex organs are found on the same plant in these sex habits.

The other type of allogamous mating occurs between flowers on different plants and is called xenogamy. Xenogamous pollination is greatly facilitated by certain sex habits. All dioecious species are necessarily xenogamous, along with female individuals of gynodioecious species. Complete, synchronous interfloral dichogamy should facilitate xenogamous pollen transfer, as long as some plants are protandrous and others are protogynous. This is the case in heterodichogamous *Grayia brandegei* (Pendelton 1988). The degree of relatedness between mating plants is also important in determining the number of novel gene combinations possible in progeny that are products of xenogamous crossings.

Many species experience fertilization as a result of a mixture of pollinations from both self and outcross pollen. Although the term selfing refers only to fertilization between pollen and ovules with identical parents (hence dioecious plants cannot self), the term outcrossed pollen only identifies pollen from a different plant. In reality, outcross pollen may be genetically similar to the ovule it fertilizes, or it may be very different, depending upon the genetic structure of the population and pollen dispersal mechanisms. Facultative xenogamy describes species that have mixed mating systems, as opposed to the term obligate xenogamy, describing species that only outbreed (Cruden and Lloyd 1995).

Dichogamy also plays a role in the ability of self pollen to be successful. Intrafloral protandry and protogyny differ in their ability to prevent the landing of self pollen upon a stigma. Particularly when self pollen is incompatible to the stigma, excess self pollen may clog the stigma and prevent the germination of outcross pollen (e.g. Bertin and Newman 1993). Protandry may prevent, or at least decrease, selfing and stigma clogging, as most pollen is likely to have been spent prior to emergence of the stigma. However, protogyny does not accomplish this task and if the stigma is receptive at pollen dehiscence, the potential for autogamous pollination remains. An analogous situation occurs with interfloral dichogamy where an overlap in sexual phases within a plant allows geitonogamy, especially when stigma receptivity precedes pollen dehiscence (Darwin 1877, Bertin and Newman 1993).

It is important to keep in mind that the ultimate importance of a mating system rests upon the degree of genetic variability generated. It has been postulated that the importance of sexuality is also based upon the creation of novel gene combinations through meiosis, recombination and independent assortment (Ghiselin 1988). It could be argued that plant mating systems act as an alternate control mechanism for generating genetic variability. Due to the fact that plants are sessile, their environment has a disproportionately great effect on the evolution of life history traits. The control of genetic variability may have strong consequences on the opportunity for sessile plants to find favourable niches. Hence, selection for mating system characteristics may be strong.

In addition to the spatial and temporal aspects of sex organ arrangement, self-incompatibility (SI) is also crucial to determining the outcome of a pollination event, particularly between related gametes. Gametophytic SI refers to the failure of fertilization as a consequence of the haploid pollen genome. Sporophytic SI refers to the failure of fertilization as a consequence of the genome of the diploid pollen parent. Additionally, both

sporophytic and gametophytic systems can be subdivided into homomorphic and heteromorphic systems. In the former, all morphological types are identical, while in the latter there are different classes of plants within a species of different morphological forms. Included in heteromorphic SI systems are distylous and tristylous species (Barrett 1988).

## **Maternal and Paternal Influences on Seed Fitness**

### **Sexual Selection**

Sexual selection was first described by Darwin (1977) and has two main components. The first involves competition among the members of one sex for access to members of the opposite sex. Typically in nature, males compete for access to females. The second component involves one sex making a "choice" to mate with a particular individual or individuals of the opposite sex, or to otherwise 'enable' fertilization. Usually, it is females who do the choosing (Willson and Burley 1983, Silvertown and Lovett Doust 1993). Sexual selection may have an important influence on plant mating systems, either through male-male competition or female choice (Willson and Burley 1983, Lovett Doust and Lovett Doust 1988). It has been implicated as a mechanism in the evolution of dioecy (Bawa 1980, Freeman et al. 1996).

One way in which male-male competition can manifest itself in plants is through pollen competition. In addition to morphological attributes, such as pollen size (Willson and Burley 1983), there are several physiological traits which are potentially important when pollen grains compete for access to an ovule. For example, variation in the ability of pollen to disperse to a stigmatic surface, or to germinate and produce rapid pollen tube growth, and to penetrate and fertilize ovules, all enable selection to occur among males (Willson and Burley 1983, Mulcahy et al. 1983). In *Geranium maculatum*, pollen competition occurs among members of different pollination events, as well as among simultaneously deposited grains (Mulcahy et al. 1983). Female choice may also occur at different stages in the reproductive process. Females regulate the success of a potential pollen donor through their stylar and stigmatic environment (Bertin 1988). Furthermore, selective seed abortion following multiple seed set can act as a significant element of female choice (Willson and Burley 1983). Thus, potentially, sexual selection

may affect many seed characteristics, including seed set and seed vigour via competition among paternal gametes, and by maternal choice.

### **Outcrossing / Selfing in a Population**

Mating systems are characterized by the relative extent of outcrossing and selfing in the population. However, they are not easily estimated, due to the difficulty in following pollen transfer, and determining paternity conclusively, though techniques such as paternity exclusion analysis and maximum likelihood procedures are showing promise (Bertin 1988). In contrast, it is relatively straightforward to determine compatibility.

Mating systems are also dependent upon sex habit. Sex habit affects the likelihood of outcrossing events, by affecting the distance of gender components (i.e. compatible flowers) within or between plants. As a result, the potential for particular pollinations such as selfing and outcrossing between flowers, is also affected. For example, a plant cannot fully self-fertilize if it is strictly dioecious, though the products of successful sib pollinations may constitute a source of inbreeding.

Furthermore, the relative level of selfing or outcrossing may depend upon the existence and integrity of self-incompatibility (SI) systems. Thus, although it is difficult to directly measure natural outcrossing rates in the field (but see e.g. Stevens and Bougourd 1988), indirect estimates of outcrossing and selfing potential pose considerably fewer logistical problems to the interested researcher.

The performance of selfed and outcrossed products can be compared at many stages of the plant life cycle. Most often, a decrease in the fitness of progeny is expected with selfing, based upon evidence of the detrimental effects of inbreeding (see Jain 1976). The mechanism of this decline in fitness can be traced to both prezygotic and postzygotic sources. SI acts relatively early in the reproductive process, affecting pollen-stigma interactions soon after contact is made. Alternately, elements of inbreeding

depression (an outcome of the seed's genetic constitution) may become manifest later on, and can be expressed during any phase of a plant's life cycle, postzygotically.

I am interested in factors that affect the success of a particular pollination, as well as the mechanisms through which these factors are expressed. For example, what is the potential for selfing in *Thymelaea hirsuta*? If it occurs, does selfing result in a fitness reduction for the resulting progeny and what mechanisms might be acting to facilitate this fitness decline?

### **Prezygotic Mechanisms**

There are three basic stages during the pollination and subsequent fertilization process when pollen may be affected and consequently, fail to produce seed. These include: 1) the time of arrival on a stigma; 2) during the time of pollen tube growth, when the tube is growing down to the ovule; 3) at the time of fertilization. Successful pollen germination on the stigmatic surface depends upon the compatibility of the sporophytic pollen donor, and the stigmatic environment. Thus, the failure to germinate is often attributed to sporophytic SI (de Nettancourt 1977). The cessation of pollen tube growth in the stylar environment is a characteristic of gametophytic SI, based upon the interactions of pollen and style (de Nettancourt 1977). Gametophytic fitness through pollen tube growth is reflected later on, in sporophytic fitness and seedling growth (Ottaviano et al. 1980). Late-acting SI is ovarian in nature and pollen tube rejection takes place either shortly preceding or shortly after ovular penetration. Members of the Asclepiadaceae seem prone to late-acting ovarian SI (Wyatt and Broyles 1994). Moreover, incompatibility is not always complete and may contain substantial amounts of variation (O'Neil 1994).

Sexual selection may also act prezygotically. In *Turnera ulmifolia*, Baker and Shore (1995) assessed paternity after carrying out mixed

pollinations of self and outcross pollen. After determining ovule abortion rates for each treatment (pure self, pure outcross, mixed), and finding no difference among them, Baker and Shore (1995) concluded that pollen competition must play a major role in determining successful pollinations. In contrast to more typical results (Jain 1976), they found that self pollen did significantly better than any outcrossed treatments (Baker and Shore 1995).

Many studies have reported that prezygotic mechanisms are responsible for detrimental effects in selfers due to slower pollen tube growth. Using pollen-tube staining techniques, Krauss (1994) reported that all pollen tubes from self pollen in *Persoonia mollis* failed to reach the ovary, whereas 50% of pollen tubes from outcrossed pollen were observed directly in the ovary. However, this differential was not found at the half-style mark, suggesting that self, outcross, and pollen from natural pollinations did not demonstrate any significant differences in pollen tube vigour until after travelling half-way to the ovary (Krauss 1994).

In six species of Brazilian *Vochysia*, Oliveira and Gibbs (1994) also determined SI through rejection of self pollen tubes in the midstylar region, though all pollen germinated equally well, suggesting that a gametophytic SI system is at work. In *Geranium caespitosum*, although self pollen was as successful as outcross pollen in germinating, the growth rates of self pollen tubes were significantly lower than for cross pollen (Hessing 1988). Abortion rates between the two crosses were equal, indicating that a prezygotic mechanism caused the differences among self and outcross pollinations (Hessing 1988). Aizen et al. (1990) found that in *Dianthus chinensis*, self pollen had a slower growth rate than outcross pollen. Moreover, this discrepancy was exaggerated when both self and outcross pollen were placed simultaneously on different stigmas, within the same pistil. This result suggests that competitive interactions within the style may affect pollen tube growth (Aizen et al. 1990). In populations of *Aquilegia caerulea*, Montalvo (1992) found that 10% more pollen tubes reached the

ovary in outcross situations than in selfing. In milkweeds, fruit set is typically very low (< 5%) due to stigma clogging by incompatible self pollen (Wyatt and Broyles 1994). Although this self pollen germinates and penetrates ovaries at the same rate as outcross pollen, the ultimate success of selfed embryos (seed set) is extremely low. An ovarian SI mechanism is suspected to contribute to this result (Wyatt and Broyles 1994). Thus, there is ample evidence that in many species the mechanism for differential pollen success is largely prezygotic. Moreover, there is evidence for SI acting through pollen tube growth, as well as differential ovary penetration.

### **Postzygotic Mechanisms**

Postzygotic mechanisms may act at a number of stages to affect progeny fitness. The two main postzygotic mechanisms producing lower fitness in selfed progeny are seed abortion and inbreeding depression. Seed abortion is controlled by the maternal parent and is known to affect seed set in many species (Willson and Burley 1983). As a postzygotic mechanism, it acts subsequent to fertilization. Based on the level of mutational load, inbreeding depression can also affect progeny fitness at any stage following embryo formation.

#### **a) Seed abortion**

*Geranium caespitosum* did not undergo differential success in fruit initiation among cross types although later embryo abortions were significantly more frequent (20%) in geitonogamously pollinated flowers (Hessing 1988). This resulted in a decreased seed set produced by geitonogamy over pure outcrossing. Furthermore, selfed seeds have a lower seed mass, being only 85% the mass of outcrossed. The overall fertility of selfed flowers (measured as percent of mericarps producing seeds) was significantly lower (selfed were 42% of outcrossed) than that of outcrossed flowers (Hessing 1988). In *Geranium maculatum*, outcross treatments

produced an increase of 100 to 300% in seed number over self treatments (Ågren and Willson 1991). In distylous *Jasminum fruticans*, 'pin' morphs are capable of selective embryo abortion though 'thrums' are not (Thomson and Dommée 1993).

Bertin and Sullivan (1988) examined the extent of SI in *Campsis radicans*, a species they previously considered self-sterile. They found that when self and cross pollen were applied simultaneously, there was a reduction in fruit production, compared to a treatment using inert talc plus cross pollen in the same proportion. Self and outcross pollen tubes were found to grow at the same rate, thus the possibility of physical blocking on the stigmatic surface, and stylar interference in pollen tube growth (both prezygotic mechanisms) was ruled out. Interestingly, up to one third of all seeds from self + cross mixed pollinations were sired by self pollen. This phenomenon was termed 'cryptic self-fertility' (Bertin and Sullivan 1988), as opposed to 'cryptic SI' (Mayer and Charlesworth 1991) where mixtures of self and cross pollen yield adverse effects to otherwise viable self progeny.

It has been suggested that the production of excess flowers in *Salix myrsinifolia-phylicifolia* is related to the ability to selectively abort genetically inferior offspring (Elmqvist et al. 1988). More specifically, Rigney (1995) investigated the incidence of ovule abortion in *Erythronium grandiflorum*. When both outcross and self pollen were applied to the stigma simultaneously, more inbred seeds aborted than did outcross seeds. Paternity of aborted ovules was determined using allozyme electrophoresis. Rigney (1995) argued that postzygotic abortion mechanisms may act to increase average offspring quality by eliminating seeds with larger mutational loads and lower genetic quality; the remaining seeds would also have access to a greater proportion of the available resources.

Guitian (1994) found evidence in *Prunus mahaleb* in agreement with the Selective Abortion Hypothesis. This hypothesis centres around the

production of excess flowers so that plant can 'decide' which fruits to carry through to development (see also Elqvist et al. 1988). In contrast, Baker and Shore (1995) observed no differences in ovule abortion rates among selfed, outcrossed or mixed pollination treatments in *Turnera ulmifolia*.

#### **b) Inbreeding depression**

Attempts have been made to dissect the early- and late-acting stages during which inbreeding depression affects a selfed seed, or subsequent stage of plant development. Johnston (1992) stated that inbreeding depression was "a proportional decrement in fitness of selfed relative to outcrossed progeny" (pg. 689). In his investigation of progeny fitness in *Lobelia cardinalis* and *L. siphilitica*, Johnston (1992) found that cross type did not significantly influence seed number per fruit, or seed mass per fruit although there was always a nonsignificant increase in seed weight associated with outcrossed seeds. The life cycle was partitioned into the following components: 1) seed maturation to germination; 2) germination to six weeks; 3) six weeks to flowering, Year 1, and 4) subsequent flowering in Years 2 and 3 for field populations only. In greenhouse studies, significant levels of inbreeding were found during the seed maturation to germination stage only. In the field, one population (of three studied) was found to suffer significantly during the second year from inbreeding depression measured in terms of reduced fertility. Overall, however, by the end of the first year's reproduction, inbreeding depression accounted for a reduction in net fertility of 54% in *L. siphilitica*, and 43% and 71% for two populations of *L. cardinalis* (Johnston 1992). Thus, although these species are completely self-compatible, and the effects of inbreeding depression are only expressed subsequent to seed maturation, the later stages of seed growth demonstrate high levels of inbreeding depression.

Similarly, Parker et al. (1995) measured progeny fitness of selfed treatments in *Epilobium angustifolium* and *E. ciliatum* by determining seed

number produced per fruit, percent germination, and mature plant biomass. For *E. angustifolium*, all three life cycle stages showed significant detrimental effects of selfing while *E. ciliatum* only demonstrated a significant decline in biomass (Parker et al. 1995). Thus inbreeding depression due to selfing may manifest itself in many different stages of the life cycle of a plant.

Delesalle and Muenchow (1992) detected inbreeding depression in *Sagittaria latifolia* where geitonogamous pollinations are possible in monoecious morphs of this species. Seed set was greater for outcrossed matings in all populations and germinations was significantly reduced in three (of four) populations studied.

In *Thymus vulgaris*, a gynodioecious species, zygotic lethality through inbreeding depression from hermaphrodite selfing was suspected to be reducing selfed progeny fitness (Assouad et al. 1978). The seeds of females (obligate outcrossers) have an increased rate of germination compared to hermaphrodite seeds, which can self (Assouad et al. 1978). In fact, a substantial amount of self-fertilization is thought to occur in the wild during open pollination in hermaphrodites.

Karoly (1994) compared inbreeding effects in two species of *Lupinus*. The progeny of *L. nanus*, a more outcrossing species, suffered greater effects of inbreeding than did *L. bicolor*, the more selfing species. It was suggested that inbreeding depression will affect outcrossing species more than selfers, since they have already been relatively purged of deleterious recessives.

In *Epilobium obcordatum*, simultaneous application of self and outcross pollen reduced seed set to a level comparable to when only self pollen is applied. No differences in germination in pollen tube growth were found but there was a negative correlation between seed set and inbreeding coefficient (Seavey and Carter 1984). Thus, inbreeding depression may often be confounded with maternal choice.

## **Secondary Sex Characters**

**Sexually monomorphic (or 'homoecious') plant species (i.e., those where all individuals are of similar gender phenotypes) are clearly different from sexually polymorphic species. In the latter, individuals of different gender phenotypes differ in details of sex habit and often also in details of secondary sexual traits. Lloyd and Webb (1977) described secondary sex characters as "all differences between males and females in structures other than androecia and gynoecia" (pg. 180). For example, plant size, growth rate, vegetative reproduction, survival, habitat distribution, reproductive effort, flowering phenology and other morphological features have been found to differ between sexes (Lloyd and Webb 1977). Only sexually dimorphic (or 'heteroecious') populations can diverge with respect to secondary sex characters and, as a result, this phenomenon has been largely examined in dioecious populations. Lloyd and Webb (1977) suggested two main mechanisms for the development of intersexual differences: 1) that they are physiological byproducts of the genetic or developmental aspects of sex determination, or; 2) they are traits that have evolved in response to particular selection pressures and have adaptive value.**

**Most authors attempt to explain secondary sex characters in an evolutionary context (see Bierzychudek and Eckhart 1988 for a review). For example, in the dioecious *Silene latifolia*, female morphs have lower photosynthetic rates and stomatal conductance (Gehring and Monson 1994). They appear to reallocate more nitrogen towards developing fruits, away from leaves, resulting in decreased photosynthesis. Furthermore, females may be under stronger selection than males to conserve water, especially later in the growing season when soils are dry and fruit development is occurring. This hypothesis is supported by the finding that females have higher photosynthetic water-use efficiency and lower stomatal conductance to conserve water (Gehring and Monson 1994). In a gynodioecious species**

of the same genus, *S. vulgaris*, that occasionally produces gynodioecious individuals, there are differences in nectar content between hermaphrodite and female flowers (Jolls et al. 1994). Hermaphrodites produced more sugar per flower than females, a result that is difficult to explain if females are expected to be pollen-limited and need to encourage pollinator visits (Jolls et al. 1994).

Sexual morphs can also be differentially affected by the same conditions. Havens et al. (1995) examined the effects of changes in the level of nutrients on male and female allocation patterns in *Abutilon theophrasti*. They found that female allocation varied in terms of the number of flowers initiated, number of locules, percentage fruits matured, and percentage of ovules matured. In contrast, male reproductive components, measured as pollen size, anther size and stamen numbers did not differ with nutrient level. Maternal allocation was more plastic than paternal allocation in its ability to respond to nutrient changes (Havens et al. 1995). The ability of a plant to reallocate its maternal resources on a number of levels should be particularly advantageous when resource levels are uncertain (Lloyd 1972). In a similar study, *Cucurbita pepo* was found to be affected by nitrogen level (Lau and Stephenson 1993). Most measures relating to female reproduction were significantly affected by nitrogen treatments. However, only two measures of male reproduction were influenced; number of pollen grains per staminate flower and mean pollen grain size (Lau and Stephenson 1993). Thus, several authors have tried to interpret secondary sex characters in an evolutionary context.

### **Spatial Segregation of the Sexes (SSS)**

Lloyd and Webb (1977) have stated "there are indications that in several sexually dimorphic species, the sexes do not have identical niches" (pg. 189). They used as evidence Darwin's (1877) observation of greater frequencies of females in dryer sites in gynodioecious *Thymus serpyllum* and

Dommée's (Dommée pers. comm. as cited in Lloyd and Webb 1977) observation that poor, rocky soils with open vegetation favoured hermaphrodite individuals.

In a more recent review, Bierzychudek and Eckhart (1988) gave several proximate mechanism that could produce SSS: 1) differential mortality of the sexes; 2) sex choice; 3) the ability of females to skew their progeny sex ratio; 4) habitat selection through directed propagule dispersal or vegetative growth; 5) differential germination requirements of the sexes. To determine that SSS is an evolved trait, it should be demonstrated that the species must "possess a mechanism for generating habitat assortment by sex" (Bierzychudek and Eckhart 1988, pg. 37).

Lovett Doust and Cavers (1982) investigated the sex ratio in 11 populations of *Arisaema triphyllum*. These were found to range from male- to female- biased. However, females were more prevalent in high pH, high nutrient sites with less shading. By performing reciprocal transplant experiments, they also showed that 'sex choice' was dependent entirely on the current environmental conditions of the plant (Lovett Doust and Cavers 1982). Not only did they demonstrate SSS, but provided strong evidence of a mechanism, suggesting that it was an evolved response.

According to Bierzychudek and Eckhart (1988), spatial segregation of the sexes (i.e., that similar sexual morphs are clustered together, more so than different ones), does not necessarily indicate niche partitioning. These authors suggest that the term niche partitioning only refers to the situation where intersexual competition pressures are greater than intrasexual competition pressures. If sexual segregation was favoured by selection to decrease intersexual competitive pressures then niche partitioning exists. Thus, the term 'niche partitioning' refers exclusively to habitat segregation of the sexes due to intersexual competition, which, according to Bierzychudek and Eckhart (1988) has never adequately been shown to exist. They "urge that future studies of the spatial distributions of male and female plants (and

of any other ecological features regarding the sexes) avoid inferring that SSS is adaptive or represents an involved response to competition between the sexes, until confirmatory evidence is obtained" (pg. 41).

In most cases, the causal mechanism for SSS relates to differences in reproductive biology among the sexes (Lloyd and Webb 1977, Bierzychudek and Eckhart 1988) and not competition. Male and female fitness may be influenced differently by environmental heterogeneity, creating a more or less favourable habitat for one sex or the other. Female morphs have often been found associated with less physically harsh habitats than males. This association is explained by the larger reproductive investment that females make in the development of ovules. Thus, potentially, their resource demands are greater than males.

In *Rumex acetosella*, female plants were taller than male plants (Harris 1968). Detrimental effects on growth due to environmental factors were greater in female plants than male plants. In an andromonoecious shrub, *Aesculus pavia*, Bertin (1982) found woodland sites had < 2% bisexual flowers while in field sites, 5 - 40% of flowers were bisexual. Light availability was cited as the most important factor influencing floral sex ratio in *A. pavia*. Moreover, among inflorescences on the same plant, bisexual flowers had a tendency to occur where light was most abundant (Bertin 1982).

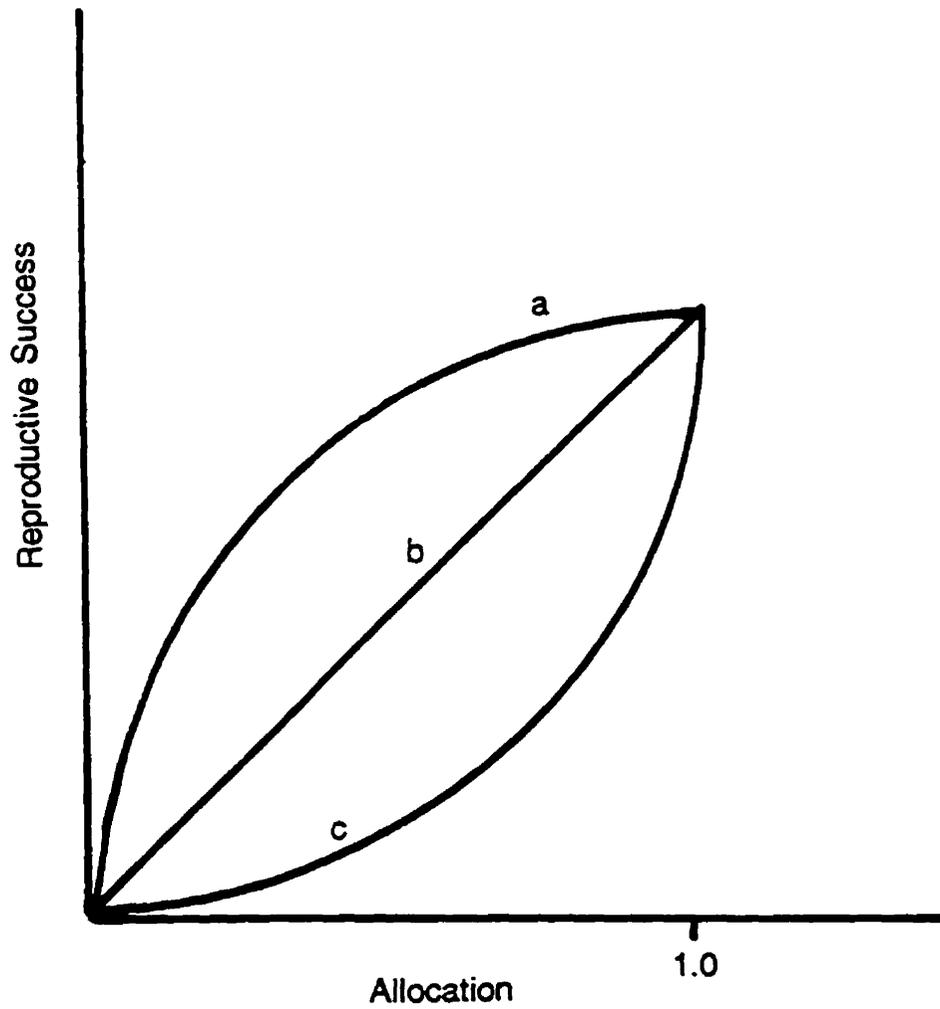
Water availability is also an important resource, and sex ratio has often been found to correlate with a moisture gradient. For example, in *Silene alba*, moister areas have more female-biased sex ratios (Lovett Doust et al. 1987). Freeman et al. (1976) examined five dioecious wind-pollinated species (*Acer negundo*, *Ephedra viridis*, *Atriplex confertifolia*, *Thalictrum fendleri*, and *Distichlis spicata*) in arid and semiarid habitats. For all five species, there was a significantly female-biased sex ratio at less saline and lower elevation (higher moisture) sites. These authors suggested that both male and female fitness was increased as a result of habitat separation.

**Females are moisture-limited during fruit development, after pollen has already been shed, and pollen dispersal is more effective when plants are at higher elevations (Freeman et al. 1976). Furthermore, the floral sex ratio on monoecious plants is also affected by moisture level (Freeman et al. 1981).**

## The Evolution of Dioecy

The primary force driving the evolution of dioecy has long thought to have been the avoidance of inbreeding depression, i.e., selection for outcrossing (Lewis 1942, Ross and Weir 1976, Charlesworth and Charlesworth 1978a, b). Most genetic models have attempted to incorporate selfing rates and estimates of inbreeding depression (Lewis 1942, Ross 1970, Ross and Weir 1976, Charlesworth and Charlesworth 1978a, 1978b). However, more rigorous examination of this hypothesis has led to the development of ecological explanations related to sexual allocation, sexual selection theory and pollinator preference (for reviews see Bawa 1980, Thomson and Brunet 1990).

The reproductive success (fitness) through maleness and femaleness in hermaphrodites can be described algebraically as a function of relative levels of resource allocation (Charnov 1982). Resulting fitness gain curves can be accelerating, linear or decelerating (Fig 1.1). The ease with which any unisexual mutant could invade an hermaphroditic population is dependent on this function and "any process that produces an accelerating fitness gain for increased investment, whether through male or female function is a candidate mechanism for the evolution of dioecy" (Thomson and Brunet 1990, pg. 12). The conditions which describe the ESS (evolutionarily stable strategy) situation are determined by combining male and female fitness curves. If  $\alpha$  is hermaphroditic success through maleness relative to unisexual male success and  $\beta$  is hermaphroditic success through femaleness relative to unisexual female success, then the set of  $\alpha$  and  $\beta$  can be determined (Fig. 1.2) (Charnov 1982, Maynard Smith 1989). If the set is convex, hermaphroditism is the ESS as unisexual success does not exceed hermaphroditic success in all cases. However, if the set is concave, then unisexual success exceeds hermaphroditic success and dioecy is the ESS. The fitness set ( $\alpha$  and  $\beta$ ) is determined by male and female gain curves



**Figure 1.1. Fitness (reproductive success) of either sex, as a function of resource allocation; a is described as decelerating, b as linear and c as accelerating. From Charnov (1982) and Thomson and Brunet (1991).**

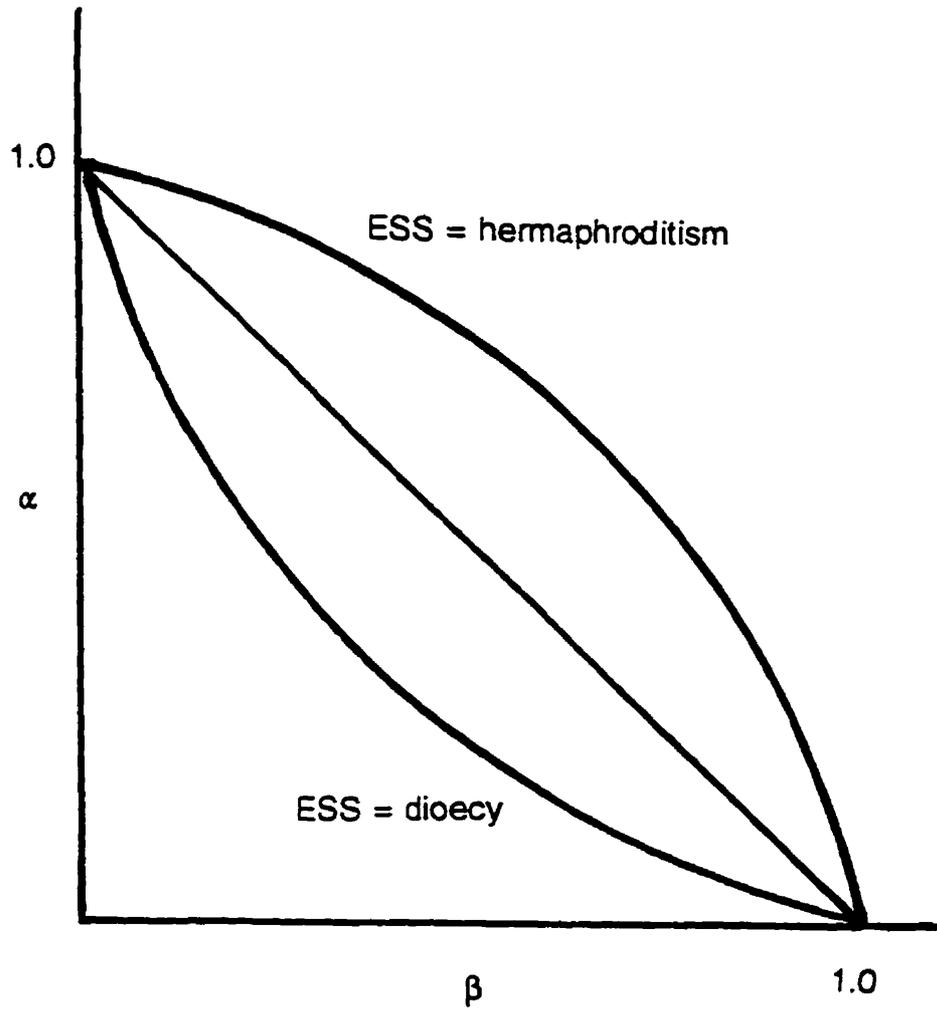


Figure 1.2. Conditions for stability of an hermaphroditic or dioecious strategy where  $\alpha$  = hermaphroditic success through male function and  $\beta$  = hermaphroditic success through female function. A concave curve defines the ESS for dioecy while a convex curve defines the ESS for hermaphroditism.

(Charnov 1982, Maynard Smith 1989) and, theoretically, any conditions or factors that affect male or female gain curves can potentially affect the evolution of dioecy.

### **Ecological Correlations**

The dioecious sex habit has been associated with the following: fleshy fruits, woodiness, tropical island habitats, large plant size, small and unspecialized, pale flowers where pollination is zoophilous (most often entomophilous), and in temperate areas, wind-pollination (Bawa 1980, 1994, Fox 1985, Muenchow 1987, Thomson and Brunet 1990, Charlesworth 1993, Renner and Ricklefs 1995 and Sakai et al. 1995). The strength and relevance of these correlations has been controversial, especially due to two main problems with analyses. First, the role of phylogeny has not been incorporated in many studies (but see Fox 1985, Renner and Ricklefs 1995, Sakai et al. 1995). Associations may arise from over-representation of particular genera with a common ancestor as opposed to any common, but independent causal mechanism (usually inferred from correlations). Second, many of these traits themselves are correlated, confounding causal effects attributed to associations (but see Muenchow 1987, Ricklefs and Renner 1995, Sakai et al 1995).

The reported correlation between fleshy fruits and dioecy (Bawa 1980, Fox 1985, Sakai et al. 1995) has been described as weak by some authors (Muenchow 1987, Thomson and Brunet 1990). Fleshy fruits are proposed to encourage an accelerating female gain curve because although animal dispersal (encouraged by fleshy fruits) is energetically more expensive, it may provide a disproportionate gain in female reproductive success, presumably through subsequent seed placement in favourable sites (Bawa 1980). Muenchow (1987) only found a 'weak' correlation after controlling for certain confounding factors such as woodiness, flower character and pollination mode. Instead, the author associated fleshy fruit more with an

understory habitat and the presence of limited dispersal agents. Muenchow (1987) did "not suppose fleshy fruit plays a causal role in the evolution of dioecy." (pg. 293). The fruits of *Thymelaea hirsuta* are nonfleshy (Tan 1980; personal observation).

There are several sources that support the correlation between woodiness and dioecy (Bawa 1980, Muenchow 1985). Woody plants are usually comprised of trees and shrubs, both perennial habits. Synchronous dichogamy may become difficult to maintain in large plants and thus selection for outcrossing favours the evolution of a dioecious sex habit (Bawa 1980, Thomson and Barrett 1981). However, Thomson and Brunet (1990) argue that the question of geitonogamy has never been adequately tested empirically. Furthermore, de Jong et al. (1993) proposed that persistent selfing would result in the purging of lethal alleles, weakening the argument that inbreeding depression can exist in combination with strong selfing, thus favouring outcrossing. However, self-incompatible species suffer increased costs, in the form of pollen wastage due to geitonogamous transfer as well as stigma clogging. These costs may select for increased separation between the sexes and hence dioecy (de Jong et al. 1993). Although size is implicated in the evolution of dioecy, the mechanism is debated. Ricklefs and Renner (1995), however, found that climbing growth (architecture) was significantly associated with woodiness. Hence, they suggested climbing growth as opposed to woodiness (and thus size) *per se* was correlated to dioecy.

There is a strong association between small, unspecialized flowers that are inconspicuously coloured white, pale yellow or pale green and dioecy (Bawa 1980, 1994, Fox 1985, Muenchow 1987, Thomson and Brunet 1990, Sakai et al. 1995). Pollinator behaviour as well as sexual selection have been invoked as causal mechanisms. The argument states that if plants are served by small, generalist insects sensitive to the number of flowers per plant as an attractant then greater flower number results in

increased pollination visits and thus increased pollen dispersal. Furthermore, female sterility would be favoured if female resources could be allocated to production of male flowers that increased pollinator attraction. Intense male-male pollen competition would cause the fitness gain accrued from pollen dispersal to exceed the fitness loss due to a decrease in ovule and seed production and result in an accelerating male gain curve (Bawa 1980, Thomson and Brunet 1990). Muenchow (1987) has stated that the type of pollinator availability in an understory habitat could lead to further correlations with traits not directly associated with causal factors of dioecy.

The specificity of pollinator behaviour combined with differences in flower number could further exaggerate the asymmetry of male and female reproductive strategies by causing a unidirectional transfer of pollen from plants with many flowers (which are more male) to plants with fewer flowers (which are more female) (Beach 1980 in Bawa 1980). Moreover, male sterility may concurrently be favoured if inbreeding depression is avoided in self-compatible plants or stigma clogging in self-incompatible ones.

The correlation between dioecy and tropical island habitats is also controversial (Bawa 1980, Thomson and Brunet 1990, Ricklefs and Renner 1995). Some argue these correlations "almost certainly have highly indirect causes" (Thomson and Brunet 1990, pg. 15) and are "especially susceptible to phylogenetic redundancy" (Thomson and Brunet 1990, pg. 15). Sakai et al. (1995) tested 'Baker's Law' in Hawaiian flora. Baker (1967 as cited in Sakai et al. 1995) stated that colonists would most likely be self-compatible hermaphrodites and Hawaiian Islands are high in dioecy due to subsequent selection for outcrossing. However, they did not find support for this notion as single-island endemic species were not associated with dioecy, as would be predicted by Baker's Law (Sakai et al. 1995).

The correlation between dioecy and wind pollination in temperate areas is likely a consequence of being woody according to Thomson and Brunet (1993). However, Charlesworth (1993) hypothesized that dioecy is

more likely to evolve if the pollination mechanism, such as wind or small insects, cannot discriminate between male and female flowers. Specialized flowers may not become part of dioecy because female fertility is likely to decline if females of dioecious species lack pollen to attract specialized pollinators. However, there are no reports of possible direct mechanisms explaining the correlation between windpollination and dioecy (Thomson and Brunet 1990).

Dioecy is likely to have arisen polyphyletically, since it is not common, but is widespread (Lewis 1942). The evolutionary time scale is well beyond the individual researcher's, and thus, attempting to determine causal factors in the evolution of dioecy is difficult because it can only be done indirectly. In fact, "the existence of ecological correlates of dioecy may not help us to infer which factors are important or unimportant in the evolution of dioecy" (Charlesworth 1993, pg. 487). We can examine correlations and propose possible mechanisms based upon these correlations but empirical studies are required to test the assumptions and expectations of a particular model.

### **Pathways to Dioecy**

Although correlations observed between dioecy and particular plant traits may indicate the presence of certain selection pressures driving a species towards dioecy, any proposed change must also have a genetic explanation. Just as the associations previously discussed imply the existence of certain mechanisms and selective pressures driving the system towards dioecy, the presence of intermediate sex phenotypes provide clues for the steps involved and can be used to infer which genetic changes are required as the population switches from an ancestral, hermaphroditic one (Lewis 1942, Richards 1986) to a dioecious one. It is important to recognize that several pathways exist in nature, relevant to different species (for reviews, see Bawa 1980, Ross 1982).

Five main pathways have been proposed and they include: 1)

heterostyly → dioecy; 2) hermaphroditism → gynodioecy → dioecy; 3) hermaphroditism → androdioecy → dioecy; 4) hermaphroditism → dioecy; 5) monoecy → dioecy. Though each step in a pathway may seem distinct, the stages for each are various degrees along a continuum and thus species evolving to dioecy may be littered with various types of intermediates. *Thymelaea hirsuta* is not a heterostylous species, and thus the first pathway will not be described.

**a) Hermaphroditism → gynodioecy → dioecy**

The pathway from hermaphroditism to dioecy via gynodioecy has two main steps. In the conversion of an hermaphrodite population into a gynodioecious one, the introduction of a male-sterile ('female') mutant is involved, possibly working cytoplasmically (Lewis 1942, Ross 1976, Charlesworth and Charlesworth 1978a). The male-sterile mutants must produce more than twice as many ovules as the hermaphrodites to be maintained in the population. However, if the hermaphrodites suffer from inbreeding depression, then this threshold is lower as male-steriles are more likely to achieve an accelerating gain curve with the help of obligate outcrossing and increased ovule production (Ross 1976, Charlesworth and Charlesworth 1978a).

In the next step, mutation at a second locus (that most likely acts as a modifier), in the gynodioecious population gradually changes hermaphrodites into males. Complete linkage of these genes results in dioecy. However, if linkage is not complete, populations containing males, females and hermaphrodites may appear (Charlesworth and Charlesworth 1978a). Intermediate stages often demonstrate relatively constant females but somewhat variable polleniferous plants ('inconstant' males with hermaphrodites). Ross (1970) has discussed how either intense selection for outbreeding, or heterozygote advantage could encourage dioecy from an earlier gynodioecious condition.

Webb (1979b) found evidence in New Zealand Apiaceae for this pathway, with an andromonoecious stage facilitating the transition between hermaphroditism and gynodioecy. Webb (1979b) has also argued that gynodioecy should be more adequately recognized as an interim stage in the evolution of dioecy by being "defined in functional terms as a breeding system involving two sexual morphs, one female and contributing genes only via ovules and the other, male and contributing genes mainly via pollen and to a lesser and variable extent via ovules" (pg. 670).

In some situations involving cryptic dioecy, noncryptic females co-occur with cryptic males that are morphologically hermaphroditic but have nonfunctional ovaries (Mayer and Charlesworth 1990). Thus, populations may be morphologically gynodioecious but functionally dioecious. Arroyo and Raven (1975) present this type of situation in species of *Fuschia* sect. *Encliandra* where seed set of hermaphrodites is low and variable. Thus many hermaphrodites were strictly pollen donors, and possible intermediate forms in the continuum from gynodioecy and dioecy.

Analogous to the gynodioecious pathway described, an androdioecious pathway could be constructed (Bawa 1980, Ross 1982). However, it is much more problematic to explain the persistence of separate male and self-compatible hermaphrodite individuals in a population (androdioecy) especially in the context of any outcrossing advantages. Furthermore, androdioecy is a rare phenomenon in the field (but see Symon 1979).

#### **b) Hermaphroditism → dioecy**

Lewis (1942) first proposed evolution of dioecy directly from hermaphroditism. Linkage of male-sterility and female-sterility mutations with the appropriate dominance relationships would prevent formation of neuters and hermaphrodites. However, Ross (reviewed in Bawa 1980) has proposed a more gradual change with partial male-sterility genes and partial

female-sterility genes involves. The advantage to these partial steriles would be determined in part by a compensation effect where reallocation of partial male-sterile pollen function into ovules and partial female-sterile ovule function into pollen would occur. Along this pathway, both males and females would co-occur with functional hermaphrodite flowers (Ross 1982).

**c) Hermaphroditism → monoecy → dioecy**

In the evolution of dioecy via monoecy, conversion as opposed to sterility genes are involved that gradually alter the ratio of male to female flowers or pollen production in the evolution of hermaphroditism to monoecy (Lloyd 1973, Charlesworth and Charlesworth 1978b). During the interim, "both sexes show intermediate-sex forms" (pg. 138). Compensation is assumed as is the presence of some inbreeding depression and selfing (via geitonogamy) in monoecious individuals. Charlesworth and Charlesworth (1978b) developed a genetic model for this pathway and describe it as follows:

The achievement of dioecy by means of a series of mutations, gradually reducing male or female fertility would probably be an extremely slow process. Not only would time be required for mutations to arise with sufficient compensation effects, and be incorporated in the population, but the correct dominance and linkage relations would have to be met. Intermediate states should therefore be common . . . one could expect "females" to be more unisexual than "males" though each would show some degree of opposite-sex function (pg. 149).

Charlesworth and Charlesworth (1978b) also examined the evolution of hermaphroditism to monoecy. They proposed that it is likely that at least three mutations are involved. The first would convert some hermaphrodite flowers into females, while subsequent mutations would be involved in a decrease in female fertility to produce male flowers.

## **Plant Architecture**

### **Modular and Hierarchical Nature of Plant Growth**

Plants grow in a way that is fundamentally different from animals. Most animals grow in a determinate manner, meaning that they attain a particular size, with a distinct separation of germ and somatic cells. However, plants grow in a modular manner. They are indeterminate in their growth and any bud may become reproductive. There is no separation between germ line and soma. Moreover, because buds act as the germ, any mutations acquired will be passed along through subsequent cell divisions. It is this mode of growth that allows the large degree of morphological plasticity characteristic of plant growth, resulting in wide intraspecific differences in many features of plant architecture (Bradshaw 1965, Cox 1988, Silvertown and Lovett Doust 1993). Whether this variation is genetic or environmental can be debated, however it is most certainly mediated physiologically (Waller 1988).

Plant construction is both modular and hierarchical, consisting of a series of similar structures organized into various levels. For example, reproductively, plants consist of pollen and/or ovules contained in stamens and/or ovaries. These are found contained in flowers, flowers are produced in inflorescences and inflorescences are arranged on plants (Cox 1988). Similarly, branches at each level are smaller replicates of the main stem as well as of branches at other levels. The overall shape of trees is defined by the length of branches and levels of branches upon one another. The modular and hierarchical nature of plant growth allows them to be highly plastic in both morphology and reproduction.

Morphology refers to "the size, shape and positioning of plant parts" (Waller 1988, pg. 204). Cox (1988) also suggested that individual modules can be viewed in essence as metapopulations, since they are "repetitive self-similar structures at different levels of organization" (pg. 80). At each of

these levels, plants that are able to change their morphology can also change their overall structure and growth pattern. Clonality is one outcome of plant modularity. Like branches, ramets are repetitive structures that acquire the ability to become separate organisms.

Plant architecture can be described in a number of ways, both qualitatively and quantitatively. Early branch ordering systems were used to examine the stability of tree structure (McMahon 1976). One measure used, the bifurcation ratio, compared the number of branches on particular branch levels. Since plants are hierarchical in structure. Primary branches are defined as offshoots of the main stem, secondary branches as offshoots of primary branches, third order (tertiary) branches originate from secondary branches, and so on (Fig. 1.3). Steingraeber et al. (1979) and Steingraeber (1982) have argued that there is substantial variation both within plants and among plants based upon these bifurcation ratios.

In addition to branching patterns, several other measures have been used to describe plant architecture. To study clonality, Lovett Doust and Lovett Doust (1987) measured number of leaves, and shoots in *Rumex acetosella*. Tremmel and Bazzaz (1995) recorded maximum number of internodes, petiole length, and leaf area in *Abutilon theophrasti*, *Datura stramonium*, and *Polygonum pensylvanicum* to study the modularity of plants. Sipe and Bazzaz (1994) examined branch number, leaf number as well as total leaf area to characterize architecture in *Acer pensylvanicum*, *Acer rubrum*, and *Acer saccharum*. Kohorn (1994) recorded leaf size, internode length, and number of branches per internode to describe the morphology of *Simmondsia chinensis*. To compare differences in plant construction in *Apodanthera undulata*, Delesalle (1992) measured number of branches per plant, number of nodes per branch, branch length and internode length. In addition, size is generally employed to gage overall growth. However, many different measures have been used to describe

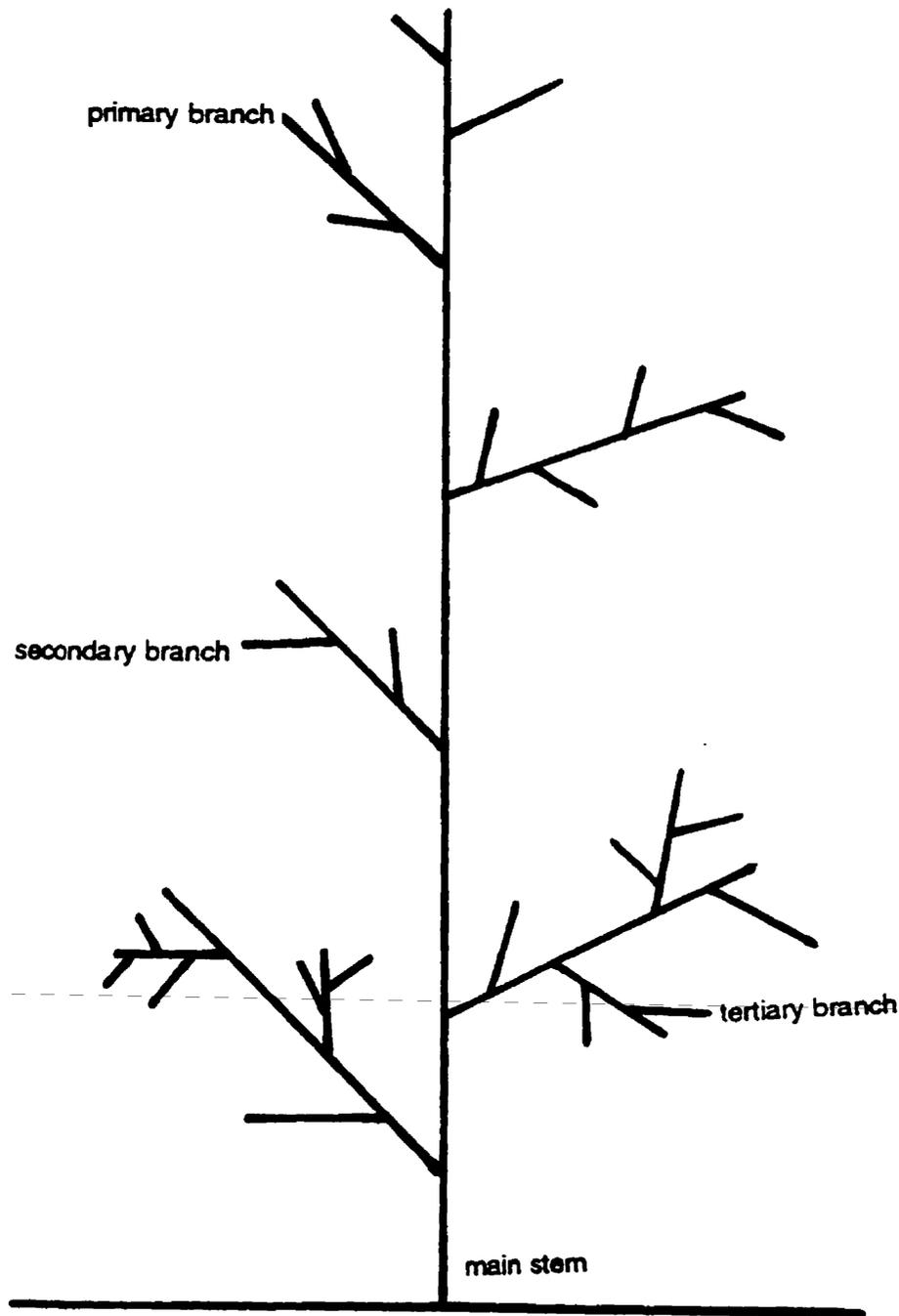


Figure 1.3. Schematic drawing of a *Thymelaea hirsuta* plant showing primary, secondary and tertiary branch orders.

plant architecture.

### **Effects of Plant Architecture**

Plant architecture influences a number of important traits that affect fitness, including growth, pollination and dispersal (Tomlinson 1987, Waller 1988). It can also provide phylogenetic information (Tomlinson 1987, Walton and Hufford 1994). Moreover, plant form can be influenced by environmental variables, providing a mechanism by which, over the long term, adaptive associations among external factors and the above traits can occur. However, morphological variation is not necessarily due to an evolved response and precise causal mechanisms for architectural variation has yet to be established for most species (Waller 1988)

Plant form may affect two important aspects of a plant, its competitive ability and its sex expression. Sipe and Bazzaz (1994) examined the effect of gaps on plant architecture in *Acer* species. In general, they found that the variation in plant form increased over the study period (3 years). Particularly in large gaps, *Acer pensylvanicum*, and *Acer rubrum* increased greatly with respect to the number of branches, number of leaves and total leaf area while *Acer saccharum* changed less so. Measurements of photosynthesis and shoot-level assimilation suggested the possibility of gap partitioning among these species. Sipe and Bazzaz (1994) "observed marked differences in architectural responses to the gap-understory gradient" (pg. 2327) among the three species.

In an experimental study on competition effects, the response of *Datura stramonium* and *Polygonum pensylvanicum* demonstrated a change in architectural response associated with neighbor species identity. Different neighbor species caused variability in resource competition and light availability mediated through changes in canopy structure. Thus plant architecture can be affected by its immediate environment and these observations demonstrate the ability of plants to exhibit varying levels of

architectural plasticity and different architectural responses, in response to their immediate environment.

The modular construction of plants has implications for reproductive biology as well. The location of meristems provides a basis for floral placement. Furthermore, the position of particular flower types on a plant may be adaptive, reflecting the preference of pollinators, fruit dispersal or fruit support strategies (Bawa and Beach 1981, Tomlinson 1987, Cox 1988, Waller 1988). For example, in the mangrove genus, *Lumnitzera*, *L. racemosa* has lateral flowers with white actinomorphic flowers pollinated by butterflies while *L. littorea* has red, zygomorphic flowers that are bird-pollinated (Tomlinson 1987). Butterflies penetrate the canopy easily while birds do not, hence *L. littorea* flowers tend to be positioned closer to the outer edges of the canopy (Tomlinson 1987).

When both male and female flowers are on the same plant but experience different resource requirements, they may be located accordingly within a plant. It has been hypothesized that the transition of the wild grass teosinte (*Zea mays* ssp. *mexicana*) into modern maize (*Zea mays* ssp. *mays*) is an example of a morphologically mediated effect. Teosinte has a main stem with many lateral branches. Each of these branches is terminated by a tassel and flanked with lateral female spikes. Iltis (1983) suggested that a mutation shortening the lateral branches caused these lateral tassels to enter a physiological area of "feminization". This morphological change resulted in a single apical tassel with lateral ears, still retaining staminate "tails" (Iltis 1983, Waller 1988).

More recently, Delesalle (1992) found pistillate buds placed at distal branch nodes while staminate buds appeared more often at early, proximate nodes in *Apodanthera undulata*. If growth conditions also affected architecture, then seasonal changes could influence sex expression through growth (Delesalle 1992). Similarly, Allison (1992) found herbivory to affect sex expression through plant morphology in *Taxus canadensis*. Deer

browsing tends to occur on larger plants and larger plants tend to be monoecious with female-biased strobilus ratios (Allison 1992).

When different flower types are located on the same plant, then their positions may be affected. Bickel and Freeman (1993) found a relationship between position and flower sex on the basis of pollination. Based on pollen dispersal principles of wind-pollinated species and the resulting fitness gain, plants of geometrically cylindrical shapes increased male floral allocation with height while more spherical plants were likely to increase female floral allocation with greater size. Thus, shifting reproductive allocation in monoecious species, may reflect architectural patterns. However, in dioecious species morphological changes at the whole plant level may occur between males and females, reflecting secondary sexual characteristics (Lloyd and Webb 1977). Lovett Doust and Lovett Doust (1987) found females of *Rumex acetosella* had significantly greater number of existing leaves, leaves initiated and shoot production than male plants.

Morphological differences among males and females may be based upon differential resource demands and there is the potential to evolve sexual dimorphism if the sexes have different optimal states (Kohorn 1994). In *Simmondsia chinensis* the gender of an individual is correlated with its architecture. Males with morphologies that are more "male-type" had more inflorescences and were more likely to flower than males that were more "female-type". Females that were structured more "female-like" produced heavier seeds than those with architecture more like males (Kohorn 1994). Fruit set was also determined to be influenced by resource availability and the author suggested the differences in morphology of reproductively more successful vs. less successful individual provided a basis to suggest "resource-based evolutionary origin of sexual dimorphism" (pg. 2384, Kohorn 1994) due to "differing morphological optima for each sex" (pg. 2390).

Ramadan et al. (1994) used plant size in *Thymelaea hirsuta* as an index of environmental quality for six Mediterranean habitats. The coastal

dune site (least arid) was determined to be most favourable, based upon crown diameter growth rate and volume. Males were larger than females at dryer, less favourable sites, such as the inland plateau. Comparison of these two habitats showed that females had greater growth rate and reproductive effort at coastal sites, while males exceeded females in both these measures at the inland plateau. This study demonstrates the possibility that reproduction and morphology are dependent upon environmental conditions (Ramadan et al. 1994). In *Thymelaea hirsuta*, the frequency of sexual intermediates is reflected in the fact that the ratio of male to female flowers varies between extremes of 0:1 and 1:0. Thus far, no investigations of architecture, or the relationship between morphology and sex expression have been conducted.

## ***Thymelaea hirsuta***

### **i) General Description**

*Thymelaea hirsuta* (Thymelaeaceae) is a xerophytic, sclerophyllous evergreen shrub, common to the Mediterranean region (Fig. 1.4). Overall, the genus *Thymelaea* contains 30 members (Tan 1980). Among them, *Thymelaea hirsuta* is located in the Section Piptochlamys Subsection Hirsutae along with *T. lythroides*, *T. languinosa* and *T. gattegosei*, all woody shrubs, semi-shrubby or suffrutescent perennials.

### **Vegetative Features**

Plant height in the field ranges from 20-150 cm, but can attain up to 3 m. El-Keblawy (1987) has reported that the rate of increase in height is greater in small plants than large ones in Egyptian coastal populations. Canopy diameters of field specimens may extend over 200 cm (El-Keblawy 1987). *Thymelaea hirsuta* is a shrub, reported to be dimorphic in its leaf and shoot morphology (Tan 1980, personal observation). The younger shoots are white-tomentose, becoming more glabrous with time. Older plants have a greatly diverged branching structure where individual branches appear pendulous in their anatomy.

The leaves are fleshy, sessile and alternate. Their stomata are distributed epistomatically throughout the leaf. According to Tan (1980) (and see Shaltout 1989), leaf margins range from slightly to fully involute. Margin shapes are orbicular-ovate, or ovate-lanceolate and sides are obtusely or acutely angled. Leaves measure (2-) 3-5 (-8) x 1.5-3 (-5) mm. Adaxial surfaces (facing the stem) are white-tomentose, while the abaxial differ slightly, being greener in colour and glabrous, appearing almost shiny at times.

The apparent leaf dimorphism does not appear to have any obvious correlates. Plants of similar ages, raised in similar situations, may have two



Figure 1.4. Drawing of *Thymelaea hirsuta*.

distinct leaf morphologies (personal observation). Tan (1980) also acknowledged a leaf dimorphism in *Thymelaea hirsuta*. The author states that the differences lie between young and old shoots on fully grown shrubs. Older, flowering branches have leaves with orbicular-ovate shaped leaf margins while branches on young, preflowering shoots have narrowly ovate-lanceolate leaves. Very mature plants in the field do not demonstrate such leaf variation, chiefly because all lower leaves may have been dropped (Tan 1980). There may also be an ecological component to leaf variation. Shrubs located at the circumference of barley and wheat fields, besides being more hardy, may be found with paler green leaves (Tan 1980). Alternatively, plants growing in exposed areas by the sea or in the desert seem to have smaller, dark green leaves (Tan 1980). In fact, I have seen both of these leaf types on the same plant. Maritime forms of *T. hirsuta* with white-tomentose flowers rather than the usual yellow-pubescent have been reported. These types also contain more slender branches (Tan 1980). Tan (1980) acknowledges the existence of ecotypes but refutes that subspecies or varietal designation is necessary. This argument is strengthened by existence of both leaf morph types on the same plant.

There are two main types of fiber in the stem of *T. hirsuta*. Bark fiber is long and narrow, with a length averaging 3329  $\mu\text{m}$  and 12.6  $\mu\text{m}$  in diameter (Shaltout 1992b). The xylem fibres have a mean length of 824  $\mu\text{m}$  and diameter of 13.9  $\mu\text{m}$  (Shaltout 1992b). These dimensions are similar to many softwoods and hardwoods employed in papermaking. Other qualities such as the low porosity suggest that *T. hirsuta* would compare favourably with bagasse and rice straw, which are more often used for this purpose.

Roots of *T. hirsuta* typically branch profusely, inhabiting two distinct soil zones and reach a depth of up to 2 m in some places (Shaltout 1987). The first zone occupies only a few centimetres just below the surface of the soil layer while the second reaches more deeply into permanently wet layers. It has been suggested that when humidity is high, as in summer months,

drops of dew may penetrate the upper layers of the soil, enabling the first root zone access to this moisture (Shaltout 1987).

Shaltout (1992a) reported the relative nutrient concentration in *T. hirsuta*. Generally, it appeared that leaves contain the highest levels of nutrients. Stem tissue contains less than leaves, and roots even less than stems. Furthermore, the highest concentrations found were of K, and the lowest Ca, decreasing in the order:  $K > N > Na > P > Mg > Ca$ . Shaltout (1992a) concluded that the highest levels of flowering and fruiting activity occurred in the spring because it is at this time that the plants had accumulated enough of the necessary nutrients to complete their reproductive cycle.

### **Reproductive Features**

Three types of flower may be found on *Thymelaea hirsuta* plants: male, female and hermaphrodite. Flowers are pale yellow, 3-4.5 mm and aggregated in axillary clusters with umbellate or subumbellate heads. Flowers lack pedicels and the inflorescence has also been described as capitulate (Dommée et al. 1995). Flower number per inflorescence ranges from 1 (personal observation) to 15 (Dommée et al. 1995). Flowers lack a corolla. The calyx constitutes the deciduous perianth which is delicate and tubular in shape with imbricate, ovate lobes that are 1-1.5 mm wide (Tan 1980). Tan (1980) has suggested that this structure may serve to attract pollinators in addition to protecting reproductive parts. As the fruit develops, the perianth is transformed into a thin, membranous fruit covering, often shed during later stages of fruit maturation.

The gynoecium in *T. hirsuta* is superior. Pistillate flowers each contain a green ovary, with a single ovule. The ovary is hirsute near the top with a translucent white style 0.7-3.00 mm long. The globular stigma appears as a slight widening of the style apex, papillate, creamy white and slightly moist when receptive. The stigma is located either just below the

perianth margin, or just above, depending upon the height of the style. Female flowers are generally more greenish in colour than either hermaphrodite or male flowers, as well as being slightly smaller in size, on average (personal observation, Dommée et al. 1990). Tan (1980) has reported that Gerber (1900 as cited in Tan) observed staminodes in female flowers. Although Tan (1980) did not personally observe these structures, I have, in a minority (approximately 2%) of female flowers. Dommée et al. (1990) also reported "tiny remains of aborted stamens" on female flowers. Small glands are located between the perianth and the ovary. These glands produce "abundant nectar" (Dommée et al. 1990). I am able to confirm the frequent presence of clear droplets of liquid situated right beside the ovary in many of the female flowers observed.

Male flowers are lemon-yellow in colour, and usually contain two whorls of stamens (personal observation, Ekler 1995, Dommée et al. 1990, Tan 1980). Each whorl contains four stamens, and thus each flower contains, on average 8 stamens. In staminate flowers, a rudimentary, nonfunctional ovary is often present (Ekler 1995). Stamen number may be variable. Tan (1980) has reported observing up to 12 stamens in a single flower at various positions. In the field, hypertrophy of stamens has been observed, and attributed to the action of an acarine parasite, *Eriophyses passerinae*. Dispersal of pollen is evident upon anther dehiscence, individual pollen grains are bright orange in colour and, together, give the anther a powdery appearance. Prior to pollen release, anthers are still orange in colour but lack the powdery appearance indicating dispersal of pollen grains. Tan (1980) also reports a conspicuous dimorphism of pollen grains.

The development of flowering is such that, after 2 weeks from the onset of anthesis, all flower types become noticeably post-receptive (pistillate flowers) or post-dehiscent (staminate flowers). Hermaphrodite flowers resemble male flowers in both colour and stature, and it is often difficult to distinguish amongst the two. Unless the stigmatic surface is

detected amongst the more showy anthers, the flower might be easily mistaken for a male.

In male flowers, post-dehiscence is signalled when petals begin turning brown. Anthers lose their bright orange colour and appear paler. Some anthers even become white. In female flowers, any liquid droplets have disappeared and the white stigma becomes brown. If fertilization has occurred and a fruit is developing, the ovary swells while still retaining its previous bright green colour. If the female flower aborts or is left unfertilized, the ovary will become yellow and ultimately brown. Both the perianth and primary reproductive organs shrivel and eventually drop off. In female flowers, it is not always clear during the final stages of receptivity, whether a fruit will develop or not. Sometimes the ovary appears to enlarge, only to ultimately become pale and fail to set fruit. Male and hermaphrodite flowers senesce and turn brown, then readily drop from the inflorescence. Development into a mature fruit requires about four weeks.

In Mediterranean field populations, anthesis occurs continuously from September to April - May (Tan 1980, El-Keblawy 1987, Dommée et al. 1990). However, under North American greenhouse conditions, plants begin to flower much earlier from July to February, with a peak from September to December. Individual floral anthesis only occurs for about two weeks for both male and female flowers. After examining many herbarium specimens, Tan (1980) reported that insects (*Cartodere* and *Macrophagus* spp.) had been preserved on the stigma of female flowers of *T. hirsuta*. These are frequent visitors to *T. hirsuta* in the field and may therefore serve as pollinators. Dommée et al. (1990) observed bees to be the main pollinators, in addition to other small insects dipterans, thrips and ants. Entomophilous pollination is in agreement with my own observations that yellow-jacket wasps were active in the greenhouse chamber where *Thymelaea hirsuta* plants were housed. The wasps moved from flower to flower, carrying pollen with them by extending their mouthparts into the

perianth tube, or resting on the anthers with their appendages. Furthermore, I observed stigmas with copious amounts of pollen on them, unlikely to have arrived there via the wind (in the otherwise closed greenhouse).

Alternatively, Ramadan et al. (1994) reported that the plant is windpollinated.

The fruit of *T. hirsuta* is dry and ovoid-shaped. The pericarp is greenish, pellicular and thin. It is easily scraped off manually. However, the epidermis of the seed (i.e. the testa) is black in colour, thick, hard and contains regular and shallow ridges. Greenhouse germination requires scarification for 10 min. with 95% sulfuric acid followed by 24 h treatment with gibberellic acid (Shaltout and El-Shourbagy 1989). Physical scarification is not sufficient to result in breaking seed dormancy. Shaltout and El-Shourbagy (1989) suggested that in the field under conditions which are semi-arid undergoing temperature and moisture extremes, germination may result. As well, the action of microorganisms might aid in breaking the seed coat.

## **ii) Sex Expression in *Thymelaea hirsuta***

The complex and hierarchical nature of plant sex expression is well-exemplified in *Thymelaea hirsuta* (Table 1.1). Recall that at the level of the flower, either a monoicous (bisexual flowers) or dioicous condition (unisexual flowers) may exist. At the level of the whole plant, sex habits may range from hermaphroditism to dioecy with a vast potential for intermediate forms. *T. hirsuta* contains an array of different sex phenotypes, reflecting variability at the level of the flower, and of the whole plant. The relative frequency of each distinct phenotype has broad implications for the mating system and patterns of sex allocation.

The potential to observe variation in plant sex expression, including *T. hirsuta*, rests largely upon two factors. The first involves how a researcher regards categorical sexual nomenclature. For example, Dommée et al. (1995) recognize only one class of females, which includes "pure" female forms (i.e., those bearing only female flowers) as well as subdioecious forms (bearing mostly female flowers but also producing a small fraction of male or hermaphrodite flowers). In contrast, El-Keblawy et al. (1995) recognize this sex inconstancy and distinguish these two types as separate categories, female and subdioecious. Secondly, the variation in sex expression observed may depend upon the time period over which the individual plants were studied, as well as the intervals between which observations were made. For example, observing the performance of a plant for several years would yield information about any between-year changes, in addition to within-year changes. These results would influence plant gender categorization. Through careful observation, Condon and Gilbert (1988) demonstrated that vines of *Gurania* and *Psiguria*, once thought to be dioecious, were actually monoecious. Clearly, lifetime reproductive schedules will be the most valuable, especially in complex sex habits. It is possible that sex expression differs among different populations of a sex-labile species.

**Table 1.1. Summary of gender phenotypes reported for *Thymelaea hirsuta*.**

| <b>Source</b>               | <b>Gender Phenotype</b>  | <b>Comments</b>  |
|-----------------------------|--|--|
| Tan (1980)                  | trimonoecious  | - functionally dioecious   |
| Shaltout (1987)             | male, female,<br>hermaphrodite,<br>monoecious,<br>gynomonoecious forms | - latter three make up less<br>than 6 %<br>- functionally dioecious  |
| Dommée et al.<br>(1990)     | male, female,<br>protandrous, and                                      | - protandrous forms are high<br>fruit producers  |
| Dommée et al.<br>(1995)     | protogynous forms  | - protogynous forms are low<br>fruit producers<br>- functionally dioecious   |
| Ramadan et al.<br>(1994)    | male, female,<br>protandrous,<br>protogynous and labile<br>forms       | - recognized gender lability<br>where some plants changed<br>their gender from one<br>reproductive season to the<br>next |
| El-Keblawy et al.<br>(1995) | 11 forms<br>(see Table 1.2)  |  |

Tan (1980) labelled sex expression in *T. hirsuta* as trimonoecious. Trimonoecy entails the production of female flowers (those bearing ovaries), male flowers (those bearing stamens) and hermaphrodite flowers (bearing both ovaries and stamens), all on a single plant. However, Tan (1980) makes the distinction between structural trimonoecy where morphologically, all three flower types are present and functional trimonoecy. In functional trimonoecy, female flowers must contribute to the next generation via ovules, male flowers through pollen, and hermaphrodite flower through both pollen and ovules. Tan (1980) concluded from his study of herbarium specimens that hermaphrodites and either staminate or pistillate flowers were rare enough to consider the species functionally dioecious and only structurally trimonoecious. Tan also noted rudimentary, apparently nonfunctional organs of the opposite sex in male and female flowers.

Shaltout (1987) examined 6 populations of *T. hirsuta*, along two 40 km transects over the course of one year. The plants were distributed along an environmental gradient of temperature and precipitation extending in a north-south direction from the Mediterranean coast of Egypt. Five sex phenotypes were recognized. Plants were categorized as male, female, hermaphrodite, monoecious or gynodioecious. However, as with Tan's (1980) herbarium-based findings, the dominant forms, accounting for greater than 94% of plants surveyed, were male or female. Thus the populations were all treated as effectively dioecious. Furthermore, the sex ratio was not significantly different from unity in four of six populations surveyed. The remaining two sites had a significantly greater preponderance of male plants (Shaltout 1987).

Dommée et al. (1990) made repeated, within-reproductive season observations on the sexual phenotype of *T. hirsuta* individuals, over the course of five years. They recorded gender phenotype for four French populations of *T. hirsuta*, and six populations along the Spanish Mediterranean coast. Four sexual morphs were recognized, fulfilling two

basic reproductive strategies, dioecy and dichogamy. Plants designated as male and female constituted the two dioecious morphs, while protandrous and protogynous forms comprised dichogamous morphs. However, within each sexual morph, there existed certain inconsistencies from what a 'pure' form might be expected to demonstrate. In fact, 'females' were strictly subgynoecious in their overall sex expression, since occasionally, a flower which produced pollen appeared on these morphs. Still, the vast majority of flowers on female plants produced only ovaries. The 'male' morph also varied and, while all males "consistently bore stamens" (Dommée et al. 1990, pg. 1455), both hermaphrodite and female flowers occasionally appeared on subandroecious 'male' individuals. 'Protandrous' individuals changed from male to female during the course of their flowering period. An overlapping transition stage could persist for up to four weeks, where male and female flowers were both present at the same time. Furthermore, the male phase of a protandrous plant contained occasional hermaphrodite flowers. Similarly, protogynous individuals could also form hermaphrodite flowers during their male phase. Protogynous plants experienced a transition period between female and male phases (where both sexes were present) for 3 to 7 weeks (Dommée et al. 1990). It is apparent that all male phases observed in the study of Dommée et al. (1990), whether in protandrous, protogynous, or in the 'male' morphs were fundamentally more variable than their female counterparts.

Dommée et al. (1990) sought to clarify the distinction between structural and functional sex expression by examining natural seed production in each of the gender morphs. Males, through their hermaphrodite flowers, clearly suffered lower fruit production than females of protandrous morphs. This result indicated that hermaphrodite flowers often lacked the ability to function as females. Protogynous individuals that produced female flowers and thus underwent fruit production earlier in the season, experienced lower levels of fruit production than protandrous

individuals that produced female flowers later on in the season. Although the amount and efficacy of pollen production in these two morphs was not investigated, Dommée et al. (1990) nevertheless conclude that since the protogynous type demonstrated lower functional femaleness and protandrous plants had greater functional femaleness (via fruit production), there is a tendency for populations overall to be functionally dioecious (Dommée et al. 1990).

It is also noteworthy that the relative frequencies of each morph obtained among the offspring of open-pollinated female and protogynous plants did not differ significantly from the overall relative morph frequencies in the field (Dommée et al. 1995). This result indicates that open-pollinated female and protogynous seeds may make a more substantial contribution to the next generation than seeds from 'male' (i.e., subandroecious) or protandrous seeds. Dommée et al. (1995) have also found evidence that maternal factors may play a role in generating skewed progeny morph ratios. Alternatively, selection pressures may be acting to cause underrepresentation of certain morphs in the offspring, from specific sexual phenotypes (Dommée et al. 1995).

Ramadan et al. (1994) studied gender phenotype and resource allocation in Egyptian populations of *T. hirsuta* for two years. They observed the same gender phenotypes as Dommée et al. (1990, 1995), while recognizing an additional sex phenotype, i.e., one that was gender-labile and "changed sex expression between seasons within a year (but differently from dichogamous individuals)" (Ramadan et al. 1994, pg. 853). Their designated boundaries between sexual categorizations were strictly delineated. 'Females' were actually subgynoecious with occasional male or hermaphrodite flowers. However, to qualify as a female, a minimum of 90% female flowers per plant was required for all survey observations. Similarly, 'males' were subandroecious with occasional hermaphrodites and females. However, on all survey visits, the proportion of males observed could not

drop below 90% to maintain a 'male' designation. Protandrous and protogynous individuals changed from male to female and vice versa respectively, as defined above. Ramadan et al.'s (1994) study was the first to note the occurrence of gender lability in *T. hirsuta*. They found from 8.5% to 20.6% of plants in various habitats exhibited gender instability.

In their report of longterm gender observations in Egyptian *T. hirsuta* populations, El-Keblawy et al. (1995) recognized eleven distinct gender groups, using records of sex expression in five natural populations, with repeated observation three to six times per year for three to six years. Categories included female, male, subgynoecy, subandroecy, stable protogyny, unstable protogyny, stable protandry, unstable protandry, dynamic andromonoecy, female duodichogamy and ambisexuality (Table 1.2). Across all populations and years, 27.8% of the plants were stable females, 7.4% were stable males, and 64.8% were individuals that could potentially contribute to the next generation through pollen and ovules, at some point in their life cycle (El-Keblawy et al. 1995). The relative frequency of stable male, stable female and labile sex forms were found to differ significantly between sites. More quantitatively, the mean gender value also significantly differed among sites. As sites differed in various environmental characteristics, the authors suggested that the correlation between sex expression and site (in particular, the male-biased ratios at dryer sites), suggested possible niche separation between the sexes (El-Keblawy et al. 1995).

**Table 1.2. Description of gender phenotypes in *Thymelaea hirsuta* according to El-Keblawy et al. (1995).**

| <b>Gender Category</b>      | <b>Description of Gender Category</b>   |
|-----------------------------|---|
| <b>FEMALE</b>               | Individuals having a gender value of 1.0 on all occasions that flowering occurred.  |
| <b>MALE</b>                 | Individuals having a gender value of 0.0 on all occasions that flowering occurred.  |
| <b>SUBGYNOECY</b>           | Individuals having a gender value $> 0.9$ on all flowering occasions.   |
| <b>SUBANDROECY</b>          | Individuals having a gender value $< 0.1$ on all flowering occasions.   |
| <b>STABLE PROTOGYNY</b>     | Individuals whose sex expression, showed a consistent female to male switch within reproductive sessions.   |
| <b>UNSTABLE PROTOGYNY</b>   | Individuals whose sex expression was protogynous except that occasional hermaphrodites noticed during male phase; stable protogyny observed $> 50\%$ of time.                         |
| <b>STABLE PROTANDRY</b>     | Individuals whose sex expression, showed a consistent male to female switch within reproductive sessions.   |
| <b>UNSTABLE PROTANDRY</b>   | Individuals whose sex expression was protandrous except that occasional hermaphrodites noticed during male phase; stable protandry observed $> 50\%$ of time.                         |
| <b>DYNAMIC ANDROMONOECY</b> | Individuals having a gender value $> 0.1$ , but having only male and / or hermaphrodite flowers.  |
| <b>FEMALE-DUODICHOGAMY</b>  | Individuals whose sex expression, consistently followed a female to male to female switch within reproductive sessions.   |
| <b>AMBISEXUAL</b>           | Sexually labile individuals not having any of the above sex phenotypes, and those which were present as one form in one reproductive session and a different form at another session. |

### iii) Evolution of Dioecy in the Thymelaeaceae

#### *Pimelea*

Burrows (1960) investigated the sex habit and seed set of several species in the genus *Pimelea*. Most species are primarily gynodioecious, however functionally a gradual progression to dioecy is observed (Ross 1970, Bawa 1980). Females of *P. traversii* produce a fruit set twice that of hermaphrodites. The ratio of female to hermaphrodite fruit set in *P. prostrata* is 10:1 and in *P. sericeo-villosa* and *P. oreophila*, it is about 20:1. Furthermore, the proportion fruit set in hermaphrodites of *P. traversii* considered alone is 41%. Whereas only 4% of *P. prostrata* and *P. sericeo-villosa* hermaphrodites set fruit while this value is 2% for *P. oreophila*. Thus, these species seem to fit well into the framework for a progression from gynodioecy to dioecy (Ross 1970).

#### *Wikstroemia*

More extensive work has been carried out on the evolution of dioecy in *Wikstroemia*, a genus poorly differentiated into species (Mayer 1991) and found among the Hawaiian flora. *Wikstroemia* has a diverse array of sex habits including hermaphroditism, morphological dioecy (regular dioecy) and cryptic dioecy (Mayer and Charlesworth 1992). Morphological dioecy entails fully functional organs of one sex in the same flower as vestigial organs of the opposite sex, making the flower unisexual. Cryptically dioecious individuals appear to be hermaphrodites but functionally pistillate flowers produce nonfunctional pollen and staminate flowers produce nonfunctional but morphologically intact pistils (Mayer and Charlesworth 1990).

The evolution of dioecy in *Wikstroemia* began with an ancestral hermaphrodite form, and progressed entirely within the Hawaiian islands (Mayer and Charlesworth 1992). Mayer and Charlesworth (1992) performed

a series of crosses that resulted in evidence for the independent evolution of dioecy on two separate occasions, once producing morphological dioecy and more recently cryptic dioecy. A gynodioecious pathway is suspected and floral traits indicate an insect pollination-mediated mechanism.

### ***Thymelaea hirsuta***

The genus *Thymelaea* contains species with widely varying sex habits. There are 15 dioecious or subdioecious species, two gynodioecious, two androdioecious, one trimonoecious (excluding *T. hirsuta* - Tan 1980), one andromonoecious, seven monoecious and one hermaphrodite. Dommée et al. (1995) have proposed that *T. hirsuta* has a breeding system consistent with an intermediate stage in the evolution of dioecy, though whether it is following a monoecious pathway, or a gynodioecious pathway is not known. In the monoecious pathway, a monoecious population would be invaded first by a dichogamous mutant of one type and then, of the opposite type leading to a stable polymorphism. Elimination of a particular phase in dichogamous individuals would create dioecy. It is also possible that heterodichogamy was preempted by the existence of duodichogamy. However, Dommée et al. (1995) do not comment on the genetics of such a system, but state there has been "almost no theoretical study of the conditions for temporal dioecy" (pg. 738).

The scenario of evolution via gynodioecy in *Thymelaea hirsuta*, according to Dommée et al. (1995), begins with an andromonoecious or hermaphrodite ancestor. Establishment of male-sterile mutants would create a gynodioecious population. Gynodioecy may even arise from hermaphroditism through andromonoecy as in New Zealand Apiaceae populations (Webb 1979b). Dommée et al. (1995) also suggested that a *T. hirsuta* ancestor may have been gynomonocious. However, once gynodioecy was established factors that select for interfloral and intrafloral dichogamy would be favoured. These same factors could cause the

development of unisexual, with complete separation in sexual function. The development of dioecy would require within-flower and within-plant unisexuality. Though Dommée et al. (1995) present both the monoecious and gynodioecious pathways as possibilities in the evolution of dioecy, they support the latter as more likely, due to the existence of more male inconstancies than female in their observations.

El-Keblawy et al. (1996a) expressed doubt that dioecy is actually evolving in *T. hirsuta* but state that "the presence of dioecy in related taxa seems to suggest that this case of *T. hirsuta* may be one of breakdown" (pg. 12). Their field evidence does not suggest a straightforward pathway via either gynodioecy, hermaphroditism or monoecy. Gender phenotypes from the field were any one of 11 gender phenotypes (El-Keblawy et al. 1995, 1996b). The presence of unstable dichogamous and ambisexual plants suggested the effects of variable female-sterility and sex conversion. As well, the presence of inconstant males and "stable hermaphrodite individuals having well-developed ovaries and producing good seed crops" (pg. 16) suggest that *T. hirsuta* is in an intermediate transition stage from gynodioecy to dioecy (El-Keblawy et al. 1996a).

A detailed examination of sexual phenotypes, including intermediate forms, of *Thymelaea hirsuta* raised in constant environmental conditions would aid in clarifying the nature of intermediate forms. With this information, a critical analysis of each pathway and how *T. hirsuta* individuals fit into the framework of specific pathways could be further examined.

## Chapter 2

### Gender Study

#### Objective

Sex expression in *Thymelaea hirsuta* is complex and variable (El-Keblawy et al. 1995). Clarification as to the extent of this variability can be achieved through a rigorous assessment of *T. hirsuta* gender, both qualitatively and quantitatively. My goal was to characterize the nature of sex expression in *T. hirsuta*, to facilitate further investigations into causes and correlations of gender and gender variability. I examined *T. hirsuta* gender qualitatively by determining its sex habit and quantitatively through the use of morphological gender (Delesalle 1989). In combination, this approach gives an assessment of spatial, as well as short-term temporal patterns in variability of gender components.

There were several reasons suggesting Egyptian field populations of *T. hirsuta* to be spatially segregated by sex along an environmental gradient related to moisture availability. Previous studies among various dioecious desert species indicated this phenomena (Freeman et al. 1976, 1981). Water is a limiting resource in desert habitats that can affect the sex ratio of dioecious species, as well as the floral sex ratio of monoecious species (Freeman et al. 1981, see also Lloyd and Webb 1977, Bierzychudek and Eckhart 1988 for reviews). Furthermore, El-Keblawy et al. (1995) found sexual phenotypes to be heterogeneously distributed with respect to site. More specifically, the less favourable, more arid sites farther from the coast were male-biased. The labile sex expression of *T. hirsuta* also presented the possibility that 'sex choice' could potentially provide a mechanism for SSS (Bierzychudek and Eckhart 1988, Schlessman 1988).

Thus, due to the fact that water is often a limiting factor in desert environments, that previous studies with other species have shown habitat segregation of the sexes on the basis of water availability, that *T. hirsuta* field surveys showed a gender ratio, more male than female at dryer sites, and the occurrence of sexual lability, it was hypothesized that spatial segregation of the sexes on the basis of water availability occurred in *T. hirsuta*. I wanted to determine if, all other factors being equal, *Thymelaea hirsuta* would become more male-biased as a consequence of limiting water availability.

## **Materials and Methods**

Seed of *Thymelaea hirsuta* was sampled from natural populations in the Mediterranean coastal region of Egypt. Maternal plants from which seed was collected originated from three main habitats: coastal dune, depression and inland. These correspond to the coastal dune, depression and inland sites described in El-Keblawy et al. (1995). These sites were all located in a warm desert climate, and the three habitat types were distributed along a north-south aridity gradient. The northern coastal dune site was characterized mainly by white calcareous rocks overlain by sandy dune formations (see El-Keblawy 1987 for details). South of the coastal dune habitat lay the depression sites. These ranged in salinity and also contained calcareous deposits. They had a water table depth  $> 1$  m in some areas. The southernmost, inland sites were characterized by calcareous sandstone, shallow soil and were the driest among the three habitat types (El-Keblawy et al. 1995, 1987; and see Shaltout 1992, 1987).

Seeds were germinated in April 1992, following scarification treatment with 95% sulphuric acid (Shaltout and El-Shourbagy 1989). Seedlings were potted three to a pot and placed on tables in the greenhouse at the University of Windsor. A subset of these became established and reached their first season of sexual maturity (i.e., flowered) two years later.

### **Irrigation Treatment**

Once established, plants were subjected to one of three irrigation treatments, assigned randomly to individuals from each habitat group. Watering treatments involved 250 mL of water / plant being applied either every 3 days, 6 days, or 9 days. As well, every thirty-six days, a fertilizer treatment of 20-20-20 NPK was applied to all plants. Occasional treatment with a general purpose fungicide, Ban-Rot (Grace Sierra Crop Protection Co., California) was also applied to protect against root rot. From the time of first

flowering in May 1994, a monthly census of flowers was carried out. For each flowering plant, the number of male flowers, female flowers and hermaphrodite flowers were recorded.

### **Gender Censuses**

At each census, flowers were recorded if they had either receptive stigmas or dehiscent pollen. Floral development in *T. hirsuta* is such that after two weeks from the time of initial flower opening, all flowers become noticeably post-receptive / post-dehiscent. In male flowers, the petals begin turning brown and the anthers become paler, losing their powdery, bright orange appearance. In female flowers, a drop of nectar, often found alongside the ovary dries up, and the creamy white stigma becomes brown in colour.

Due to the ease with which stigmatic receptivity and nonreceptivity, and anther dehiscence and nondehiscence could be recognized, and due to the monthly frequency of the surveys, I am confident that nearly all flowers were detected, and appropriately recorded. If a particular flower was surveyed in any one month, then during the next census it would be in its nonreceptive / nondehiscent stage, in which case it would not be recorded again.

### **Calculating Quantitative Gender**

Gender has a qualitative element and can be described using categorical terms. However, when a system is as variable as that observed in *T. hirsuta*, quantification is necessary for further detail and clarity. A number of researchers (El-Keblawy et al. 1995, Klinkhamer et al. 1994, Allison 1991, Delesalle 1989) have employed a standard quantitative index, one which essentially measures the degree of femaleness or maleness of a plant. I have calculated gender employing the following formula:

$$\text{Gender} = (\text{No. of female flowers} + 0.5 \cdot \text{No. of hermaphrodite flowers}) / (\text{Total no. of flowers})$$

This index gives a good indication of relative femaleness within the plant.

During each census period for which a plant was flowering, a gender value was calculated. The overall mean (GV1) was determined by summing these values and dividing by the total number of flowering periods.

Alternatively, a second mean gender value could be calculated by using the total flower production of each sex (male, female and hermaphrodite) over the course of the entire reproductive season and substituting these values into the above formula (GV2).

### **Bimodality of Gender Distribution**

To statistically test for bimodality in *Taxus canadensis* when employing quantitative gender, I used the method employed by Allison (1991). *Thymelaea hirsuta* plants were sorted on the basis of gender and assigned to one of two clusters or groups. All plants in the female cluster had a gender value of 0.50 or greater while all plants in the male cluster had a gender value less than 0.50. The between- to within-cluster ratio, B/W, was calculated with the following formula:

$$B/W = [n_F \cdot n_M \cdot (x_F + x_M)^2] / [s_F^2 (n_F - 1) + s_M^2 (n_M - 1)] (n_F + n_M)$$

where  $n_F$  and  $n_M$  are the sample sizes of female and male clusters respectively,  $x_F$  and  $x_M$  are the means of female and male clusters (using GV1 estimates) and  $s_F^2$  and  $s_M^2$  are the variance.

When  $N$ , the total sample size is  $> 8$ ,  $\ln (B/W + 1)$  is normally distributed with an expected mean of  $[-\ln (1 - 2/\pi) + 2.4 / N - 2]$  and a variance close to  $1 / (N - 2)$ . By scaling  $\ln (B/W + 1)$  and comparing it to tables for the standard normal distribution, I can test the probability that B/W is from a unimodal distribution. Rejecting the null hypothesis that  $\ln (B/W + 1) = \text{mean } \ln (B/W + 1)$  indicates that gender is bimodal and that there are two discrete gender clusters.

## **Multivariate Analysis of Variance**

A multivariate analysis of variance was performed to examine the effects of water treatment and maternal site of origin on the following variables: total flowers produced, total female flowers produced, total male flowers produced, gender value (see Calculating Quantitative Gender above), sexual lability, number of days until first flowering event and length of flowering period.

### **a) Number of flowers**

For each plant flowering in 1994 / 1995, the cumulative number of flowers produced by the end of the season was determined by summing the results of each individual monthly census. As previously stated, flower type were partitioned into female, male, hermaphrodite or neuter gender classes. For the purposes of further statistical analyses, the number of neuter flowers were discarded.

### **b) Sex lability**

Lability in sex expression was regarded as a change in plant gender over time. The magnitude of this sex change was calculated by determining a value for lability. Sex lability was quantified by summing the differences between gender values at successive censuses. Thus lability cannot be calculated for plants that produced a combination of male and female flowers but only flowered for the duration of one census.

### **c) Other measures**

Two further phenological measures were also recorded for each plant, the number of days until appearance of the first flower (recorded from the date the first flower appeared in the greenhouse) and the duration of the flowering period in days.

#### **d) Data transformation**

To better fulfill the assumptions of data normality and homogeneity of variances required for the MANOVA, most variables were transformed. Log transformation substantially improved the normality of the distribution of total number of flowers produced, total number of female flowers produced, total number of male flowers produced, and duration of flowering period. Skewness and kurtosis values were reduced and Bartlett's test for homogeneity of variances yielded a nonsignificant result for all groups following log transformation.

Days to first flowering required 'reflection' (taking the largest value + 1 and subtracting all values from it) and subsequent square root transformation due to negative skewness (Tabachnick and Fidell 1989). The normality was much improved, with a reduction in skewness (G1 = -1.205 to 0.266) and kurtosis (G2 = 1.316 to 0.563). However, there was still a significant heterogeneity in variance among water treatment groups. Nevertheless, this transformation yielded the lowest Chi Square value.

I was unable to transform gender value to fulfill the requirements of normality. For this reason, gender values were categorized into pure female, pure male and intermediate values, and coded using dummy variables. A similar problem existed for estimates of sexual lability, as a large portion of the plants that were stable had lability values of 0.0 and the distribution was extremely skewed. Sexual lability was dichotomized into plants demonstrating sex lability and plants not demonstrating sex lability (see Tabachnick and Fidell 1989). Dichotomization did not affect overall results of significance.

#### **Field vs. Greenhouse Results**

A chi-squared analysis was performed to determine whether there was a significant departure from expected frequencies of gender types, based upon field values obtained by El-Keblawy et al. (1995) and Ramadan et al.

(1994). El-Keblawy et al. (1995) found no significant differences within different sex phenotypes among years for each habitat, except among females in the inland sites. Thus expected values for the chi-square analyses were generated by taking the mean frequency of sex phenotypes over all years for each habitat. Separate analyses were performed for each habitat: coastal dune, plateau and depression sites.

### **Associations Between Maternal and Progeny Gender**

A three-way frequency analysis was performed using the SAS CATMOD application (SAS 1989) to assess the effects of maternal gender and water treatment, on plant gender. Information regarding maternal gender (seed parents of present plants) allowed placement into categories of either stable, labile or dichogamous. Water treatment levels were 3 day, 6 day, or 9 day watering frequencies. Plant gender was dichotomized into stable or labile plants. There were a total of 305 cases, and data fulfilled the assumptions for this test (Tabachnick and Fidell 1989). Tabachnick and Fidell (1989) have stated that "the purpose of multiway frequency analysis is to discover associations among discrete variables" (pg. 237) by employing frequency data in particular multi-dimensional contingency tables. One of the variables can be "considered a DV [dependent variable] and the other IVs [independent variables]" (Tabachnick and Fidell 1989, pg. 237). Thus, this is an appropriate test to determine if there are any effects of water treatment, or maternal gender on plant gender.

## **Results**

### **Floral Ratios**

During the first year of flowering, from May 1994 to April 1995, a total of 305 individuals flowered. This greenhouse population produced a total of 38,772 flowers, of which 17,175 were female, 20,671 were male, 348 were hermaphrodite and 83 were neuter (lacking sex organs). Neuter flowers were omitted from further analyses. A chi-squared analysis indicated that the ratio of male to female flowers was not significantly different from equality ( $X^2 = 322.9$ ,  $n = 305$ ,  $p > 0.05$ ).

Individual plants produced either only male or female flowers, or a combination of the two, along with occasional hermaphrodites, and neuter flowers. Of the 305 plants that flowered, 212 produced solely male or solely female flowers. Of the remaining 93 plants, producing a combination of male, female and hermaphrodite flowers, the total number of flowers of each sex differed significantly. After log normalization, a paired-sample t-test determined that there was a significant difference between the number of male flowers and female flowers produced per plant ( $t^2 = 2.00$ ,  $df = 92$ ,  $p < 0.05$ ).

### **Quantitative Gender**

Figure 2.1 shows the frequency distribution of mean gender values for each flowering individual. A significant regression between values for GV1 and GV2 demonstrated that the gender value at each census is strongly dependent upon the total number of flowers produced at the end of the season ( $n = 305$ ,  $p < 0.001$ ,  $r^2 = 0.95$ ). Figure 2.1 a) shows the frequency distribution for estimates of GV1 and Figure 2.1 b) for estimates of GV2. It is apparent that most plants were constant in their gender, with a gender value of either 1.0 or 0.0. Thirty-three percent of all individuals were "pure" or "constant" males (gender = 0.0), and 37% were pure females

(gender = 1.0). The remaining plants had an intermediate gender value.

Figure 2.2 shows the same frequency distributions for GV1 and GV2 gender values, but excluding pure males and pure females. There appears to be a tendency for plants to tend towards female-biased, or male-biased values, rather than having a unitary floral sex ratio. Rarely did a plant produce an equal number of male and female flowers, and have a gender of 0.50. This result is supported by floral ratio evidence where a significant difference between the number of male flowers and female flowers produced per plant was found (see Floral Ratios above).

### **Gender Distribution Bimodality**

The gender distribution (GV1) for all flowering plants was found to be bimodal (Table 2.1). Moreover, even without the presence of unisexuals to bias results, it is apparent that the intermediate gender values ( $< 1.0$  or  $> 0.0$ ) were also bimodally distributed (Table 2.1). This result suggests that maleness and femaleness are separated in distinct clusters, distinguishable at the 0.50 gender value level.

### **Qualitative Gender**

Plants that produced only male flowers were categorized as 'male' individuals, and plants that produced only female flowers were categorized as 'female' individuals. Quantitatively, these morphs had gender values of 0.0 and 1.0, respectively, on all flowering occasions during the 1994 / 1995 reproductive season. This constant unisexual gender strategy characterized a total of 213 plants (Table 2.2). Plants that were not pure forms, but retained a gender value of either  $> 0.5$  or  $< 0.5$  for all flowering censuses were called subgynoecious and subandroecious, respectively. Dichogamous plants were those that switched from a male sex expression (gender value  $< 0.5$ ) to a female one (gender value  $> 0.5$ ) at any time during the course of one reproductive session. With these criteria, there were 12 protogynous

plants and 13 protandrous plants. The remainder of the plants (with one exception) were duodichogamous exhibiting a double switch in gender, within a single season. In female duodichogamy, a plant begins as female, then is male, and becomes female again. In male duodichogamy, a plant begins as male, then is female and then switches to male again. There were 6 female duodichogamous plants and 11 male duodichogamous ones (Table 2.2). One plant changed gender several times and could not be classified typologically.

### **Multivariate Analysis of Variance**

The results of this MANOVA are shown in Table 2.3. Pillai's Trace value was nonsignificant for effects of water treatment and site but did indicate an overall significant interaction between water treatment and site of seed origin. There were no significant effects of water treatment or maternal site of origin on gender value (Fig. 2.3), total number of flowers (Fig. 2.4), or sexual lability (Fig. 2.6). All intermediate plants that flowered for > 1 census period demonstrated some degree of temporal gender change. The frequency distribution of sex labile individuals is shown in Figure 2.5. It is evident graphically that most plants showed a relatively small degree of lability within the single reproductive season.

Further univariate F tests resulted in only one significant variable, the number of days to first flowering (Table 2.3). Note that the number of days until first flowering, and length of flowering period only appear to differ significantly among water treatment group ( $p = 0.01$  and  $0.03$  respectively). However, the Bonferroni-adjusted level of significance is  $0.005$  to maintain an overall  $0.05$  level of significance and thus strictly, these two variables do not indicate significant differences with water treatment.

A Tukey's HSD post hoc test among all pairwise interactions (Table 2.4) showed a significant difference between values associated with the inland site when watered every 3 days, and four other site by water

treatment combinations. The identity of the latter four (subscripted with an 'a' in Table 2.3) are values from the depression site watered every 3 days, the coastal site watered every 6 days, and the coastal site watered every 9 days, as well as the plateau site watered every 6 days (Figure 2.7). Plants in the 3 day watering regime from the plateau sites flowered significantly earlier than the latter 4 combinations.

### **Field vs. Greenhouse Results**

Chi-squared analyses (Table 2.5 a, b) were performed to determine whether there was a significant departure from expected frequencies of gender types, based upon field values obtained by El-Keblawy et al. (1995) and Ramadan et al. (1994). In all three habitats, there was a significant departure from expected ratios (Figure 2.8). Clearly, a larger proportion of plants were stable in the greenhouse population than the field, and a greater proportion were labile in field populations than in greenhouse populations. Although individual plants may change their sex phenotype from year-to-year, it appears the population sex phenotypes and their frequency remains the same within any year.

### **Associations Between Maternal and Progeny Gender**

Results of the multiway frequency analysis are summarized in Table 2.5. Thus, neither maternal gender nor water treatment had a significant effect on gender. There is also no three-way association among gender, maternal gender and water treatment. The significance of the gender parameter indicates that there is a difference in frequency among levels within gender. There are significantly more stable individuals than labile but maternal gender and water treatment do not appear to influence the gender value of greenhouse plants.

**Table 2.1. Mean gender value and variance of male and female "clusters" using all flowering plants, and only intermediate gender values excluding pure forms. See text for explanation of B/W. Null hypothesis is a unimodal distribution (see Allison 1991).**

|                | <b>All flowering plants</b> | <b>Intermediate flowering plants</b> |
|----------------|-----------------------------|--------------------------------------|
| <b>Female</b>  |                             |                                      |
| x              | <b>0.94</b>                 | <b>0.79</b>                          |
| s <sup>2</sup> | <b>0.015</b>                | <b>0.024</b>                         |
| N              | <b>155</b>                  | <b>43</b>                            |
| <b>Male</b>    |                             |                                      |
| x              | <b>0.047</b>                | <b>0.14</b>                          |
| s <sup>2</sup> | <b>0.010</b>                | <b>0.016</b>                         |
| N              | <b>150</b>                  | <b>50</b>                            |
| <b>B/W</b>     | <b>16.1 *</b>               | <b>5.52 *</b>                        |

\*p < 0.001

**Table 2.2. Summary of gender phenotypes for two year old plants of *Thymelaea hirsuta* raised under greenhouse conditions.**

| <b>Gender phenotype</b> | <b>No. of plants</b> | <b>Percent of total</b> |
|-------------------------|----------------------|-------------------------|
| female                  | 113                  | 37.0                    |
| male                    | 100                  | 32.8                    |
| subgynoecy              | 20                   | 6.6                     |
| subandroecy             | 30                   | 9.8                     |
| protogyny               | 12                   | 3.9                     |
| protandry               | 13                   | 4.3                     |
| female duodichogamy     | 6                    | 2.0                     |
| male duodichogamy       | 11                   | 3.6                     |
| other                   | 1                    | 0.3                     |
| <b>total</b>            | <b>305</b>           | <b>100.0</b>            |

**Table 2.3 Results of MANOVA for total number of flowers produced, total number of female flowers produced, total number of male flowers produced, gender value, sex lability index, days to first flowering and length of flowering period in *Thymelaea hirsuta*. There is an overall significant effect of water x site interaction (Pillai's Trace = 0.155). Results of univariate analyses are also shown. Univariate probabilities have undergone Bonferroni-type adjustment for simultaneous analyses ( $\alpha = 0.005$ ).**

| Univariate F Tests                  |                | Water   | Site    | Water x Site | Error  |
|-------------------------------------|----------------|---------|---------|--------------|--------|
|                                     | df             | 2       | 2       | 4            | 296    |
| Total number of flowers             | SS             | 2.49    | 1.50    | 4.05         | 166.1  |
|                                     | F              | 2.22    | 1.33    | 1.80         |        |
|                                     | P              | 0.11    | 0.27    | 0.29         |        |
| Total number of female flowers      | SS             | 1.09    | 0.485   | 4.58         | 229.7  |
|                                     | F              | 0.700   | 0.313   | 1.48         |        |
|                                     | P              | 0.50    | 0.73    | 0.21         |        |
| Total number of male flowers        | SS             | 2.46    | 1.01    | 3.52         | 244.3  |
|                                     | F              | 1.49    | 0.61    | 1.07         |        |
|                                     | P              | 0.23    | 0.54    | 0.37         |        |
| Plant gender                        | SS             | 0.392   | 0.126   | 4.14         | 205.8  |
|                                     | F              | 0.282   | 0.090   | 1.49         |        |
|                                     | P              | 0.75    | 0.91    | 0.21         |        |
| Sexual lability                     | SS             | 0.622   | 0.061   | 0.766        | 60.89  |
|                                     | F              | 1.51    | 0.149   | 0.931        |        |
|                                     | P              | 0.22    | 0.86    | 0.446        |        |
| Days until first flowering          | SS             | 62.9    | 22.8    | 184.5        | 1990.6 |
|                                     | F              | 4.67    | 1.69    | 6.86         |        |
|                                     | P              | 0.010   | 0.19    | <0.005       |        |
| Length of flowering period          | SS             | 0.709   | 0.040   | 0.419        | 30.04  |
|                                     | F              | 3.49    | 0.198   | 1.03         |        |
|                                     | P              | 0.032   | 0.82    | 0.391        |        |
| <b>Multivariate Test Statistics</b> |                |         |         |              |        |
|                                     | Pillai's Trace | 0.054   | 0.040   | 0.155        |        |
|                                     | df             | 14, 582 | 14, 582 | 28, 1172     |        |
|                                     | F              | 1.155   | 0.857   | 1.682        |        |
|                                     | P              | 0.31    | 0.61    | 0.015        |        |

**Table 2.4. Mean ( $\pm$ SE) number of days to first flowering and the effects of water treatment and site in *Thymelaea hirsuta*. Means with the same superscript in the table do not differ significantly (Tukey HSD test with adjusted probability levels).**

| Site       | Water Treatment                      |                                      |                                      |
|------------|--------------------------------------|--------------------------------------|--------------------------------------|
|            | 3 Days                               | 6 Days                               | 9 Days                               |
| Coastal    | 135.9 <sup>ab</sup><br>( $\pm$ 8.21) | 160.9 <sup>a</sup><br>( $\pm$ 6.25)  | 166.6 <sup>a</sup><br>( $\pm$ 6.05)  |
| Plateau    | 101.6 <sup>b</sup><br>( $\pm$ 12.05) | 170.7 <sup>a</sup><br>( $\pm$ 5.54)  | 148.8 <sup>ab</sup><br>( $\pm$ 13.2) |
| Depression | 166.1 <sup>a</sup><br>( $\pm$ 7.03)  | 144.4 <sup>ab</sup><br>( $\pm$ 7.84) | 139.9 <sup>ab</sup><br>( $\pm$ 9.72) |

**Table 2.5 a) Results of  $X^2$  analyses to determine if proportions of male, female, dichogamous and labile (i.e., all other) phenotypes in *Thymelaea hirsuta* differ significantly for field (from El-Keblawy et al. 1995) versus greenhouse populations.**

| Habitat    | Chi Square | df | p       |
|------------|------------|----|---------|
| Coastal    | 34.4       | 3  | < 0.001 |
| Depression | 20.6       | 3  | < 0.001 |
| Plateau    | 16.1       | 3  | < 0.001 |

**Table 2.5 b) Results of  $X^2$  analyses to determine if proportions of male, female, dichogamous and labile (i.e., all other) phenotypes in *Thymelaea hirsuta* differ significantly for field (from Ramadan et al. 1994) versus greenhouse populations.**

| Habitat    | Chi Square | df | p       |
|------------|------------|----|---------|
| Coastal    | 61.4       | 3  | < 0.005 |
| Depression | 15.9       | 3  | < 0.005 |
| Plateau    | 15.0       | 3  | < 0.005 |

**Table 2.6. Results of maximum likelihood ANOVA from multiway frequency analysis showing separate  $X^2$  tests for each parameter, in *Thymelaea hirsuta*. No associations were significant, suggesting that neither maternal gender nor water treatment had a significant effect on plant gender. Significance of one-way factor, gender, indicates that there were differences in frequency among gender levels, labile and stable.**

| <b>Maximum Likelihood ANOVA Table</b>   |           |                   |                   |
|---|-----------|-------------------|-------------------|
| <b>Source</b>                           | <b>df</b> | <b>Chi Square</b> | <b>p</b>          |
| <b>Gender</b>                           | <b>1</b>  | <b>38.96</b>      | <b>&lt; 0.001</b> |
| <b>Gender x Maternal Gender</b>         | <b>2</b>  | <b>0.28</b>       | <b>0.87</b>       |
| <b>Gender x Water</b>                   | <b>3</b>  | <b>2.36</b>       | <b>0.31</b>       |
| <b>Gender x Maternal Gender x Water</b> | <b>4</b>  | <b>3.23</b>       | <b>0.52</b>       |

## **Legend to Figures in Chapter 2**

**Figure 2.1.** Frequency distribution of plant gender values (indicating degree of femalesness; see text) in *Thymelaea hirsuta* (n = 305). Values of 0.0 indicate plants with only male flowers; 1.0 represents plants producing only female flowers. a) GV1 values are calculated by taking the mean of gender values at each cohort; b) GV2 estimates use the total number of male, female and hermaphrodite flowers produced by a plant to calculate mean gender.

**Figure 2.2.** Frequency distribution of plant gender values in *Thymelaea hirsuta* (n = 93), omitting pure male (gender value = 0.0) and pure female (gender value = 1.0) sex phenotypes. a) GV1 values; b) GV2 values (see Fig. 1 for explanation of how GV1 and GV2 are calculated).

**Figure 2.3.** Gender value in *Thymelaea hirsuta* ( $x \pm SE$ ) for coastal dune sites (coast), depression sites (depression) and inland sites (inland) at irrigation treatment levels of 3 days, 6 days and 9 days.

**Figure 2.4.** Floral sex expression (number of female, male and hermaphrodite flowers) in *Thymelaea hirsuta* for coastal dune (coast), depression (depression) and inland (inland) sites. a) water treatment = 3 days; b) water treatment = 6 days; c) water treatment = 9 days.

**Figure 2.5.** Frequency distribution of sex lability values in *Thymelaea hirsuta*, calculated by summing the difference between successive gender values determined over the course of the reproductive session (n = 305). The high frequency of 0.0 values indicates many 'pure' or 'stable' plants in the population within one reproductive season.

**Figure 2.6.** Mean sex lability ( $\pm SE$ ) values in *Thymelaea hirsuta* from coastal dune sites (coast), depression sites (depression) and inland sites (inland). Water treatment included 3 day watering frequency, 6 day watering frequency and 9 day watering frequency.

**Figure 2.7.** Mean number of days ( $\pm SE$ ) to first flowering for *Thymelaea hirsuta* plants from a) coastal dune sites, b) depression sites, c) inland sites for 3 day, 6 day and 9 day watering frequencies. (See Table 2.5 for results of statistical analyses).

**Figure 2.8.** Comparison of frequencies of sexual phenotypes in greenhouse and field populations of *Thymelaea hirsuta*. a) coastal dune sites; b) depression sites; c) inland sites.

Figure 2.1 a)

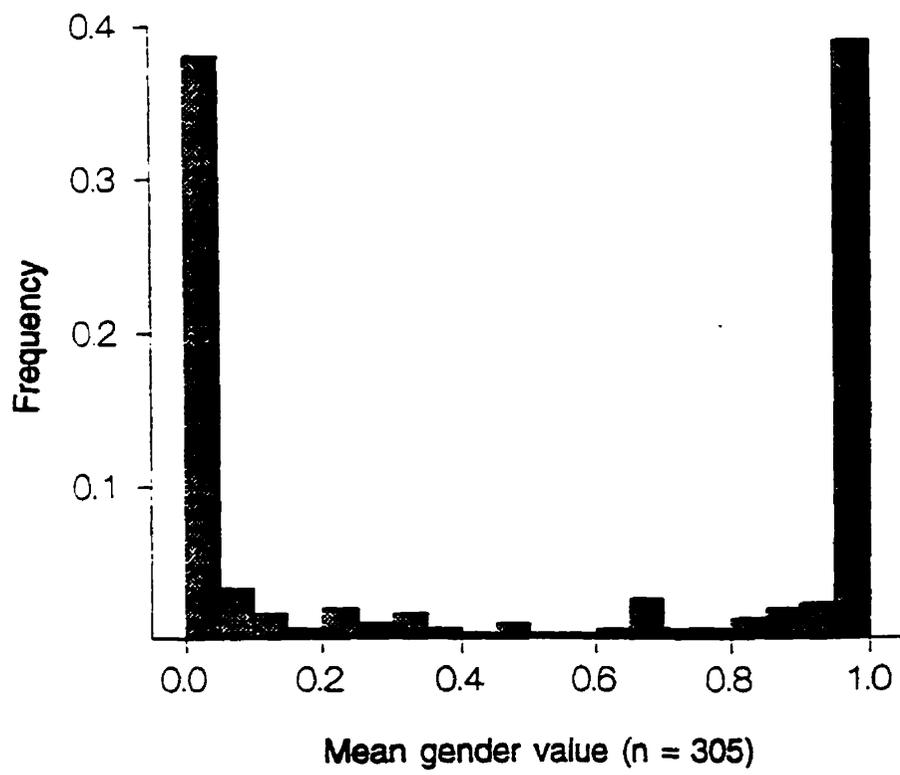


Figure 2.1 b)

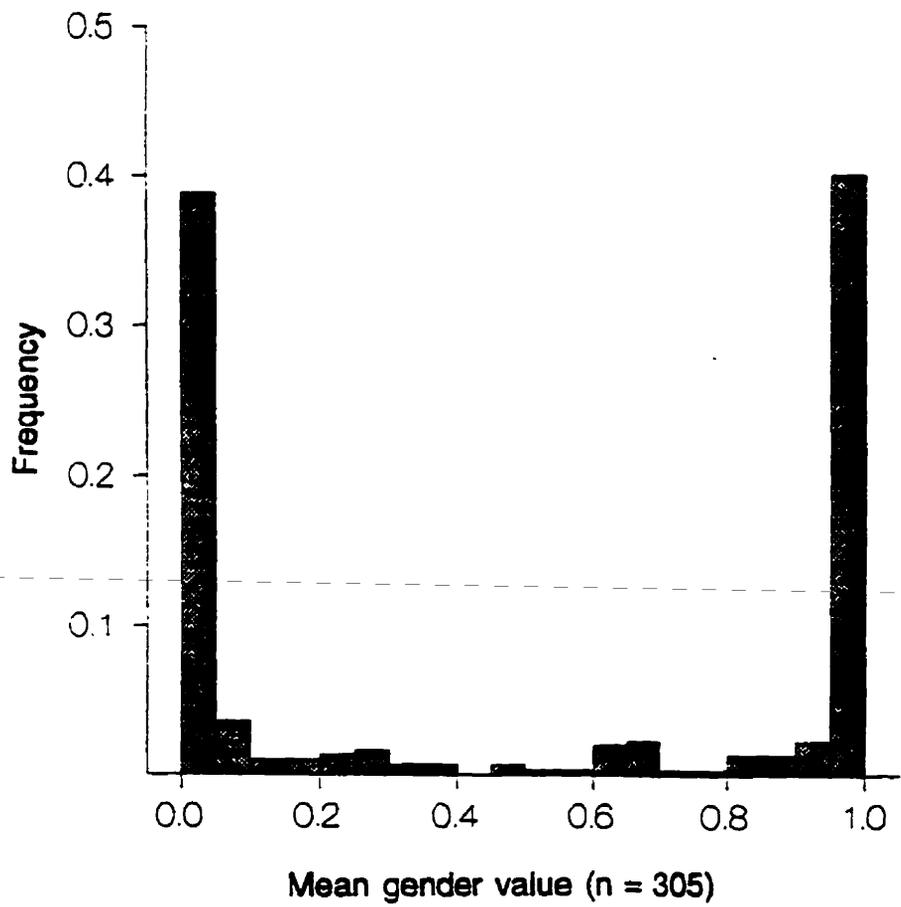


Figure 2.2 a)

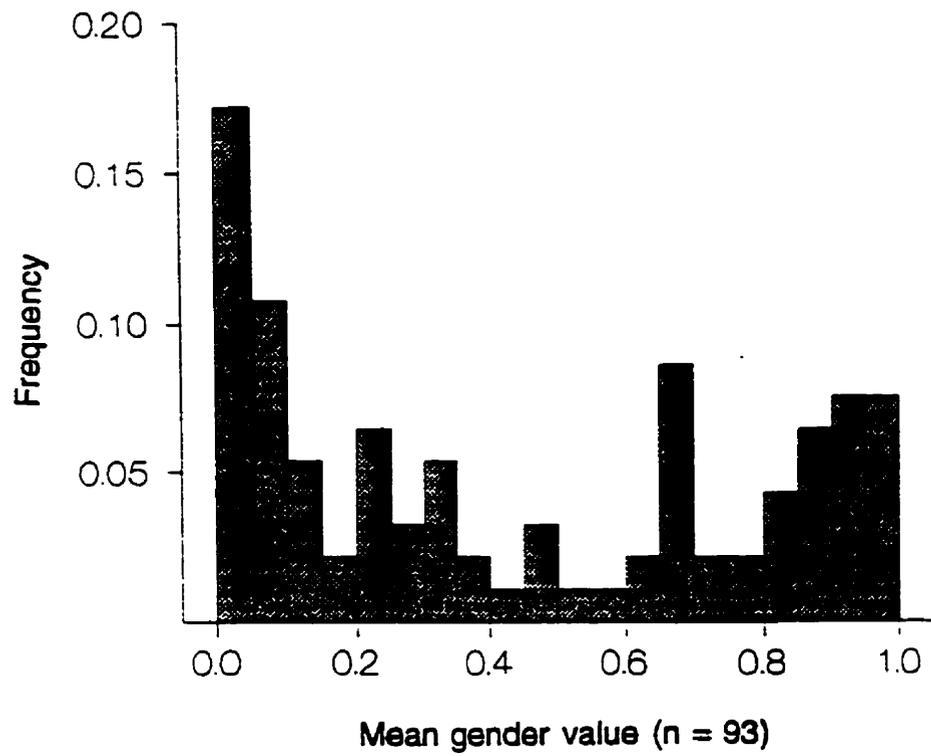


Figure 2.2 b)

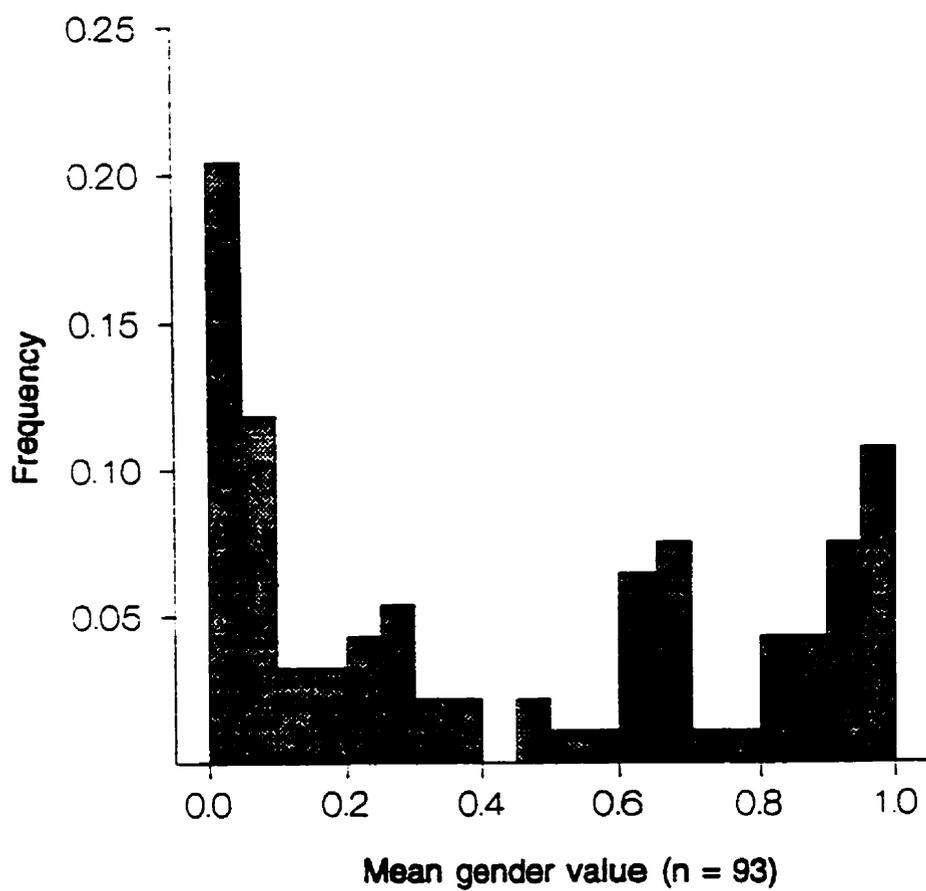


Figure 2.3

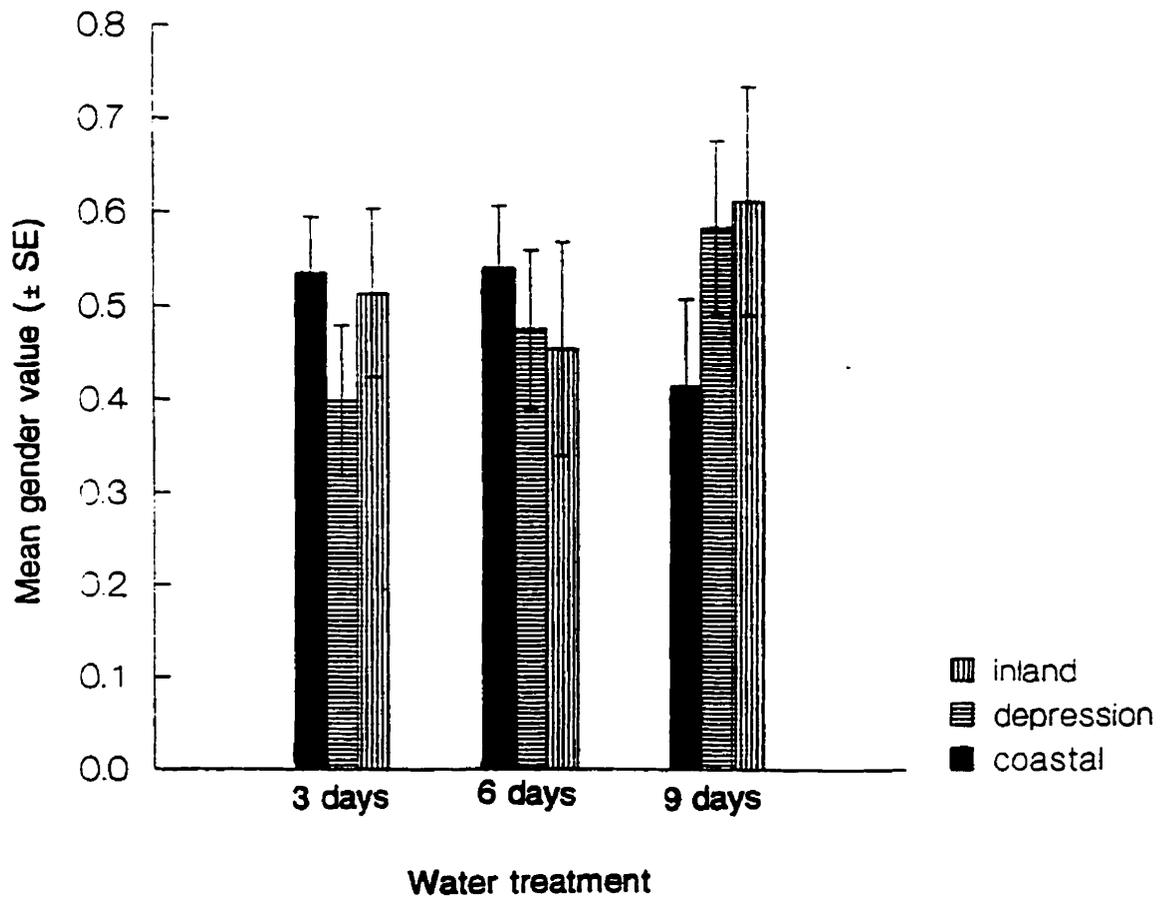


Figure 2.4 a)

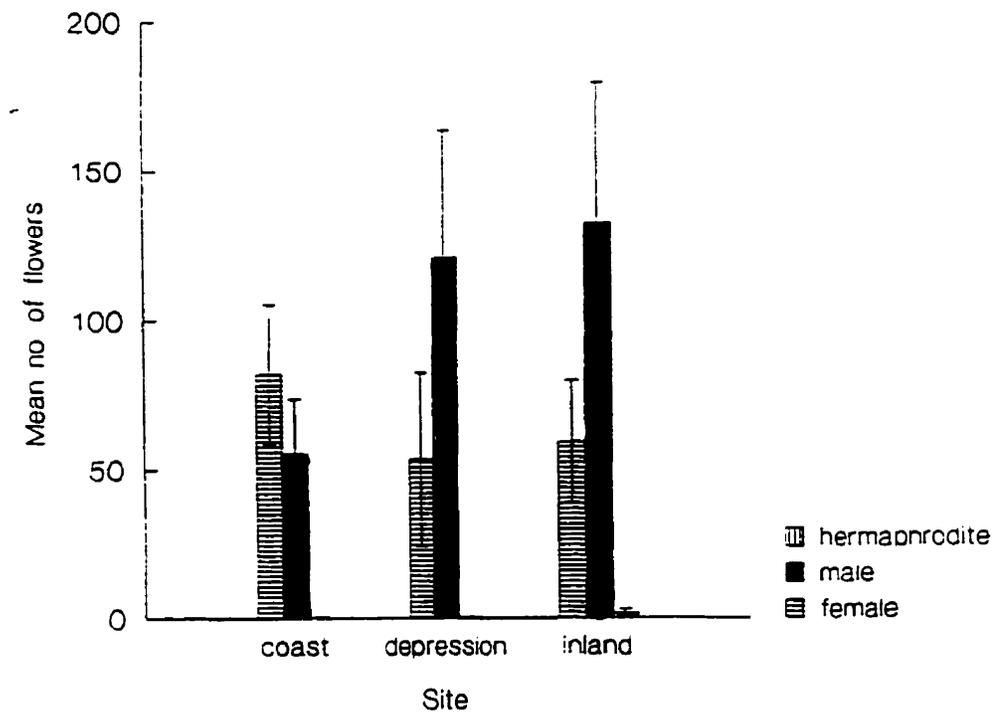


Figure 2.4 b)

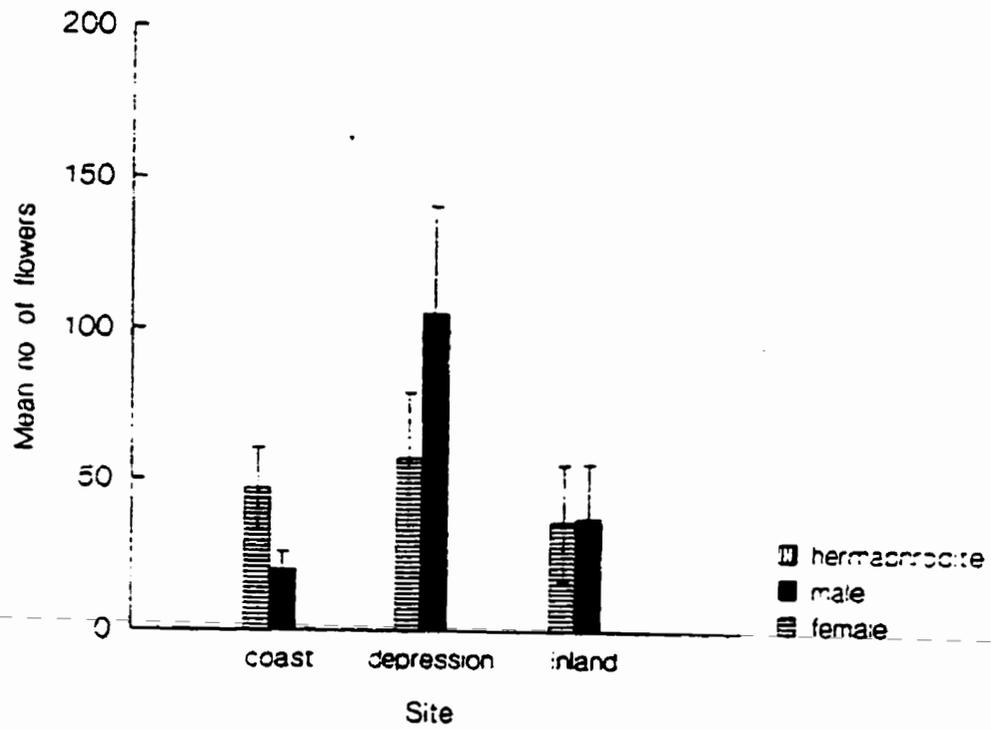


Figure 2.4 c)

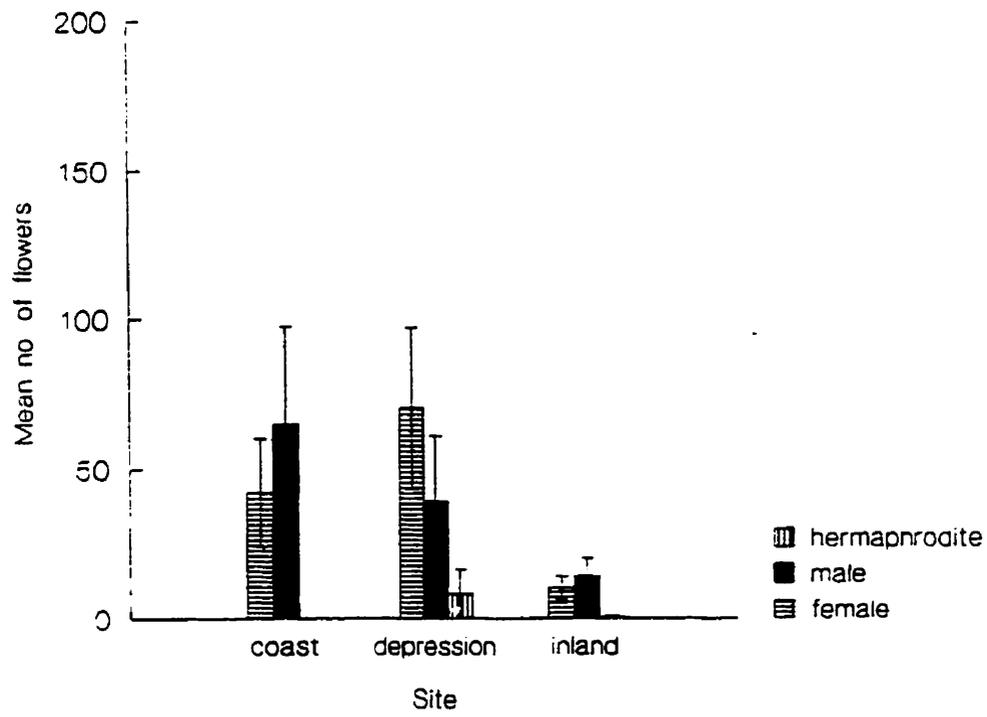


Figure 2.5

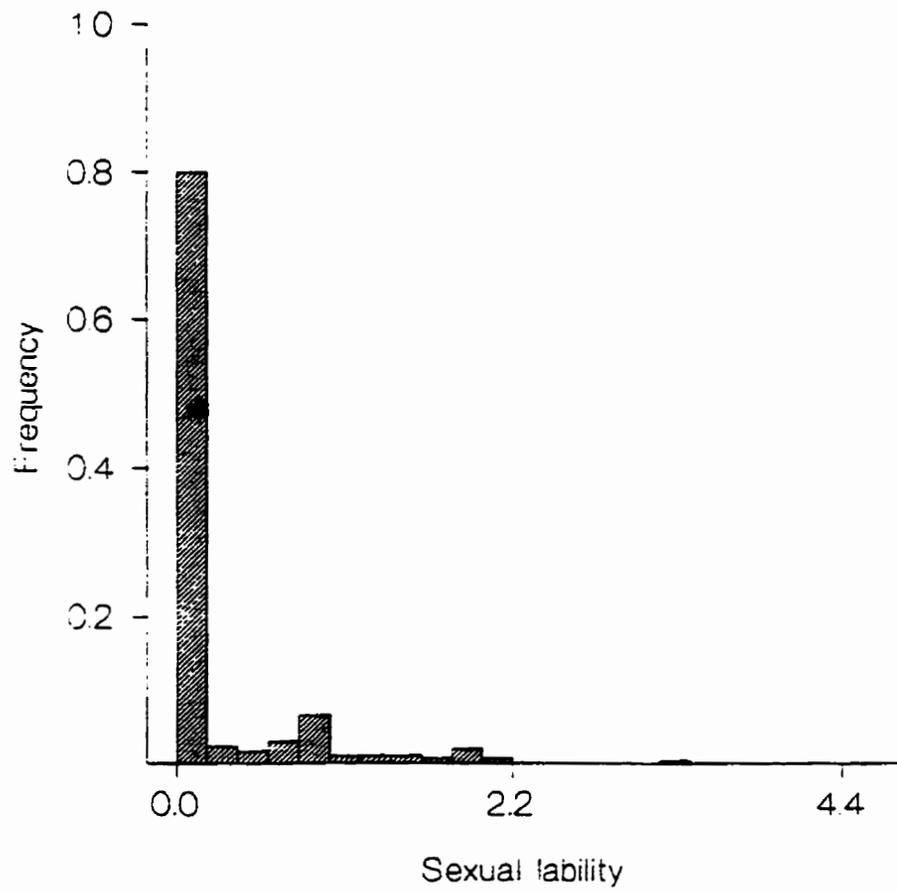


Figure 2.6

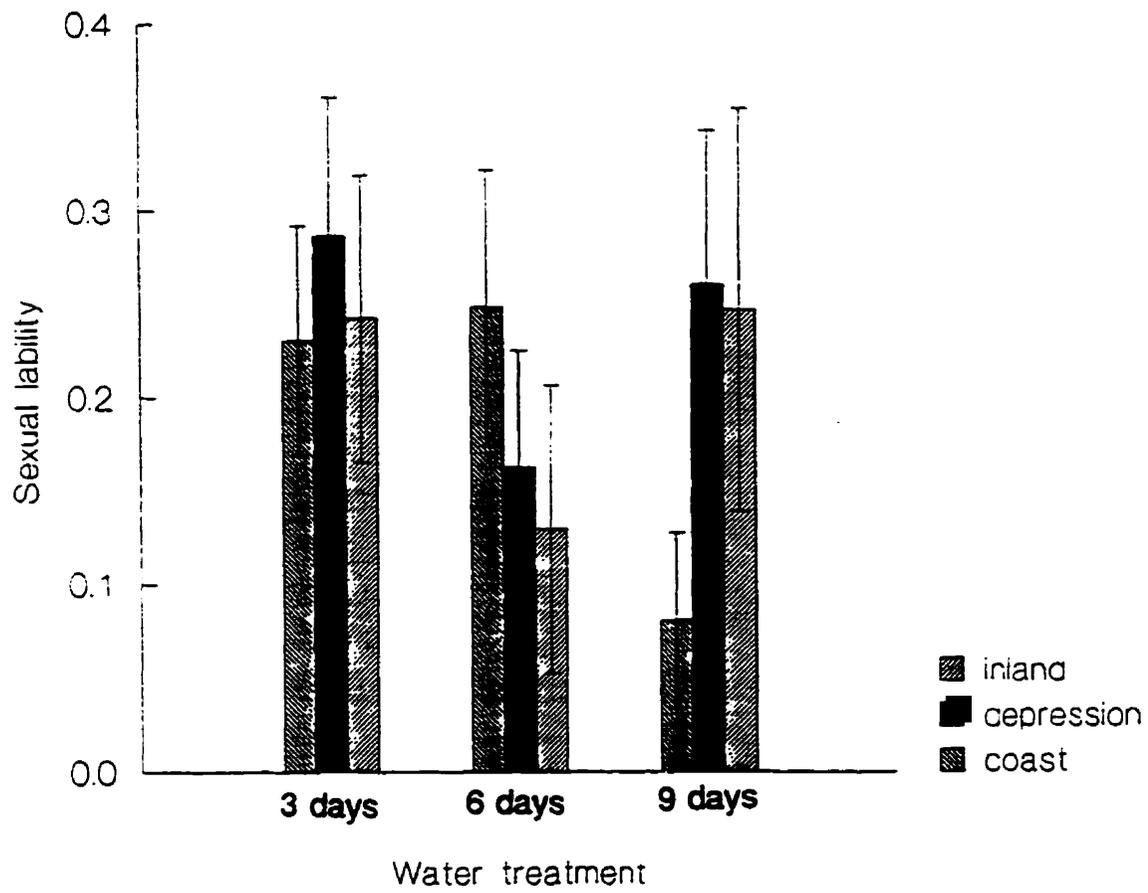


Figure 2.7 a)

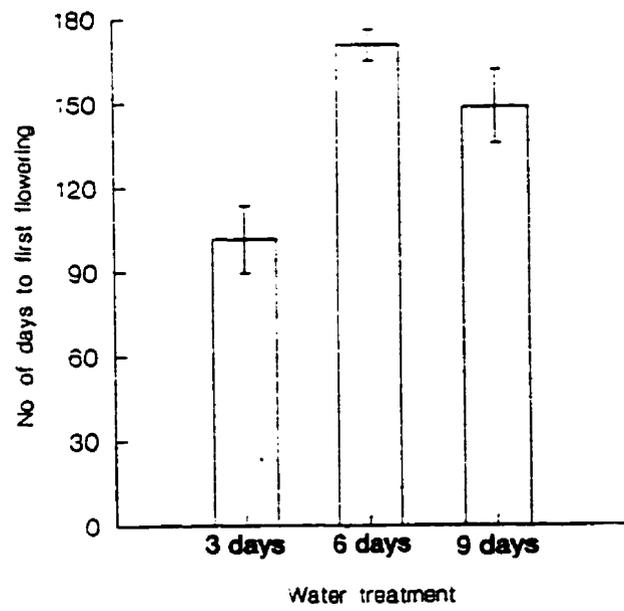


Figure 2.7 b)

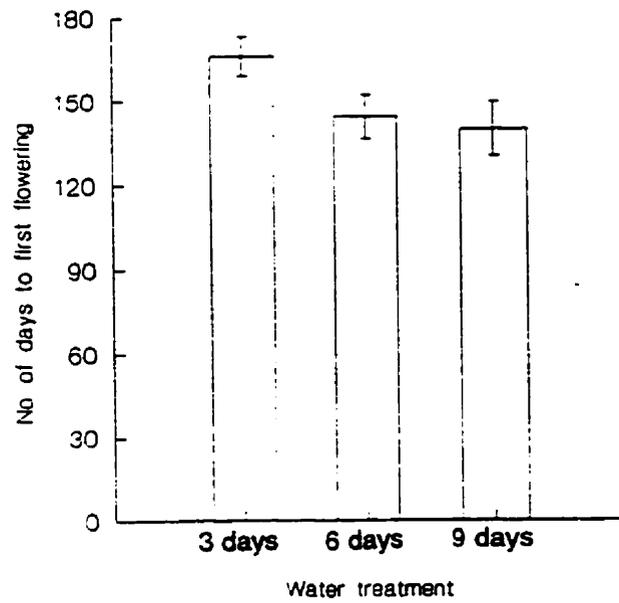


Figure 2.7 c)

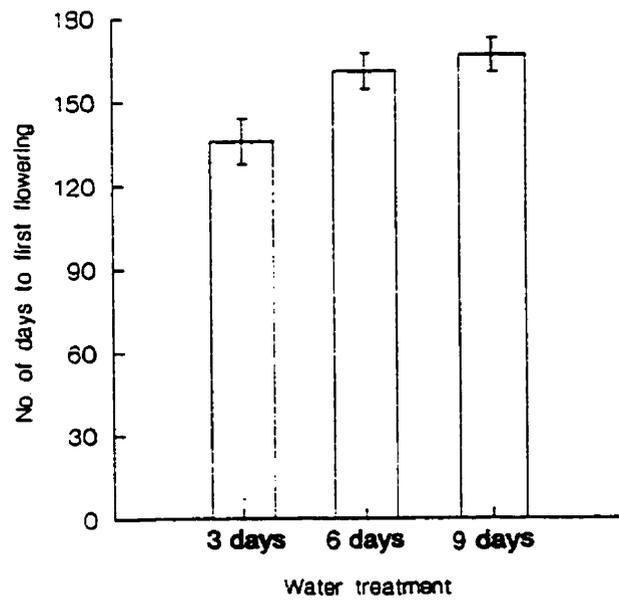


Figure 2.8 a)

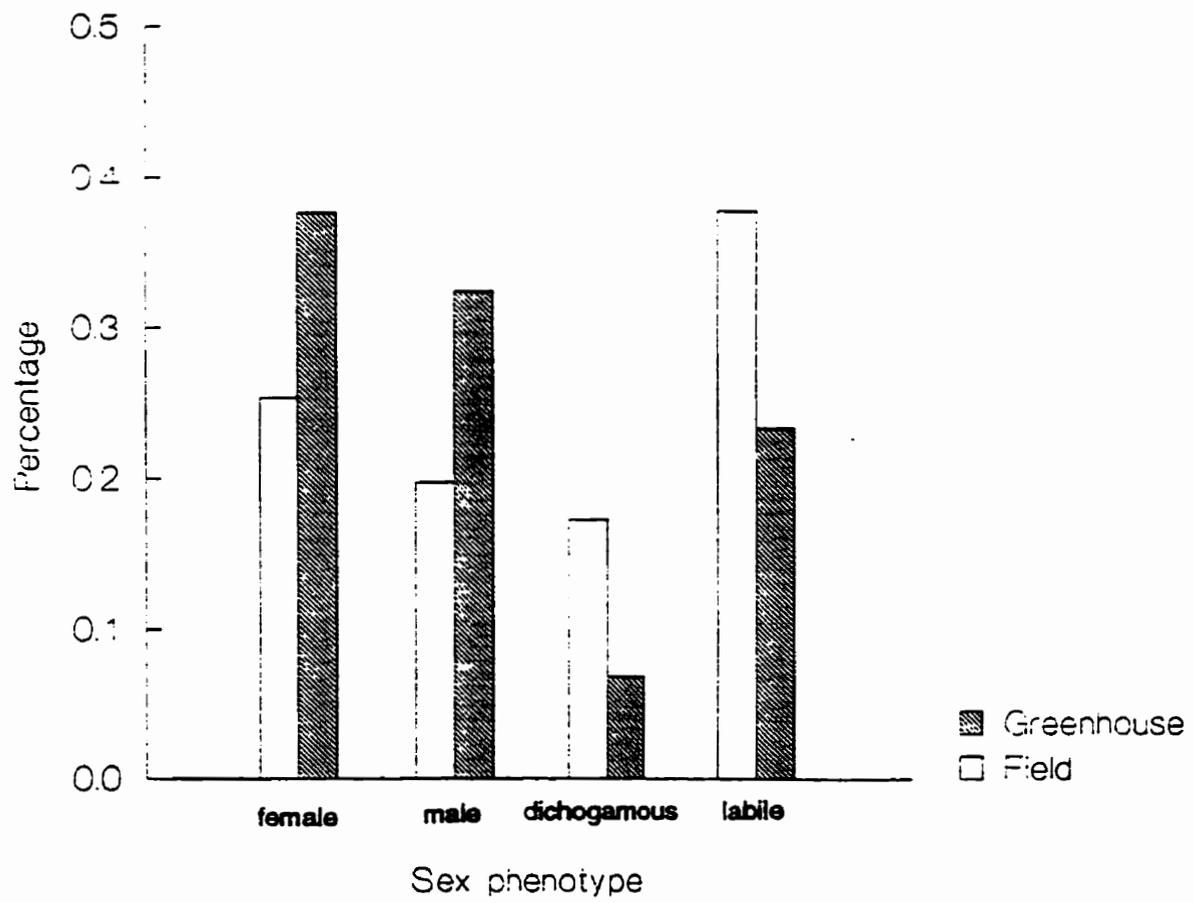


Figure 2.8 b)

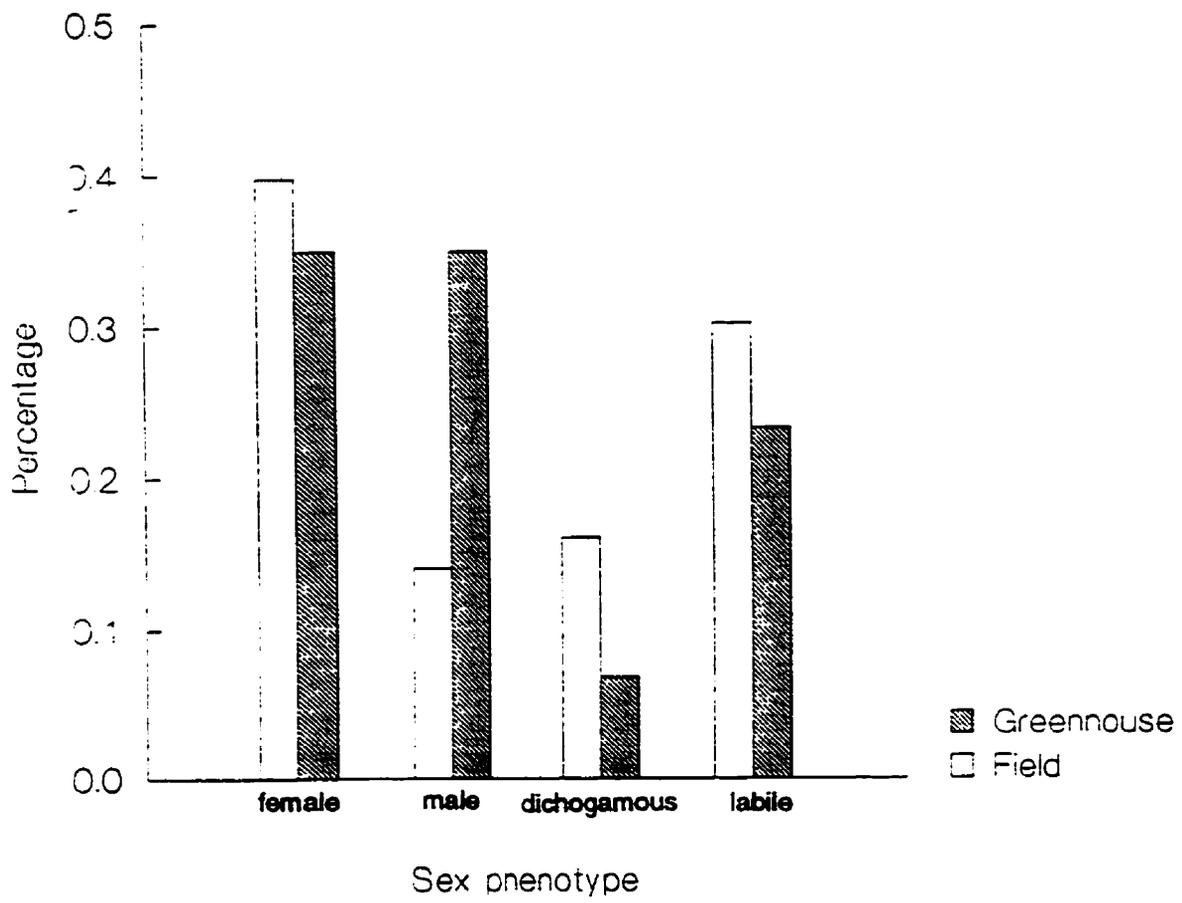
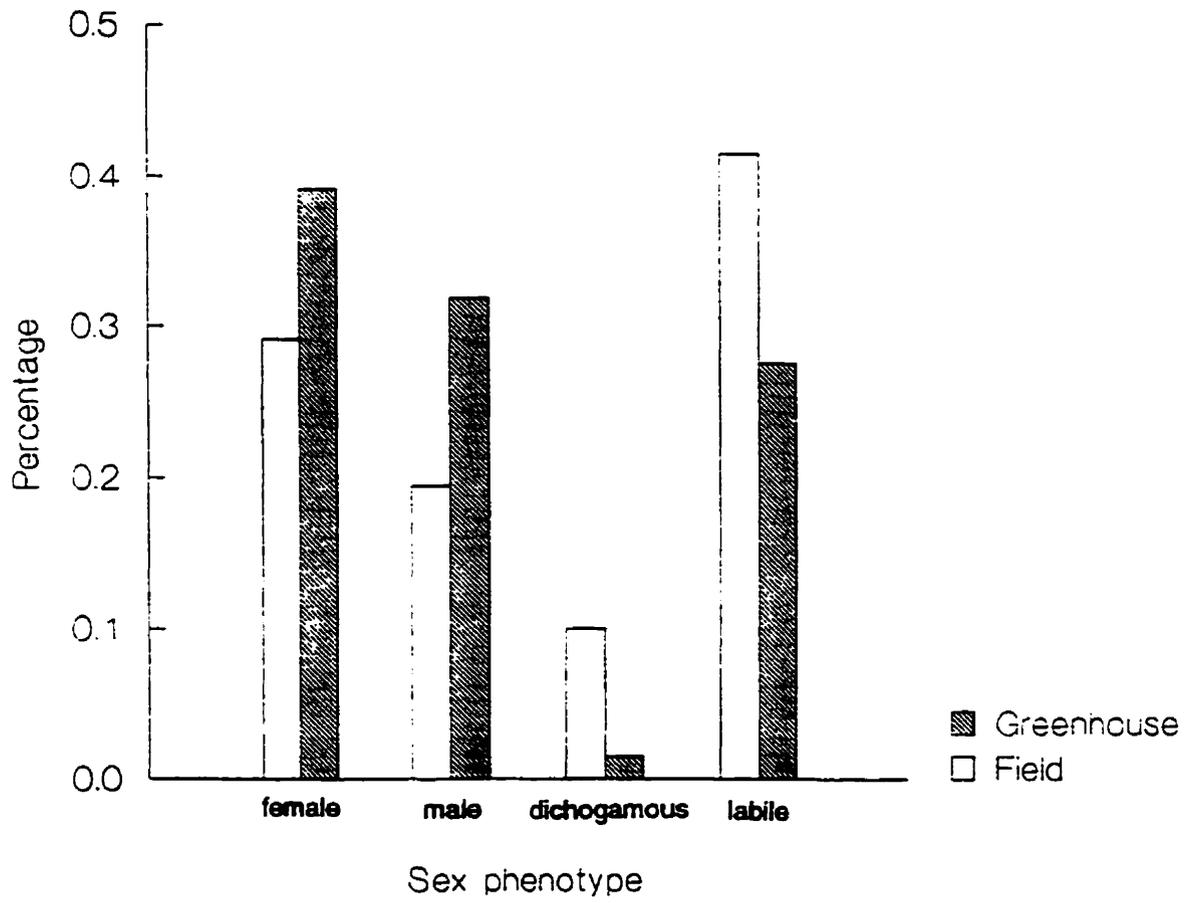


Figure 2.8 c)



## Discussion

Though historically plants have been characterized typologically according to their sex habit (Darwin 1877), the gender of a plant is essentially a quantitative phenomenon, referring to the relative success as a maternal or paternal parent (Lloyd 1979, 1980), the femaleness or maleness of an individual. Many authors have begun to investigate plant sex expression employing a quantitative approach (see e.g., Condon and Gilbert 1988, Allison 1991, El-Keblawy et al. 1995). Lloyd (1979, 1980) distinguished between the 'effective' gender of a plant reflecting actual success through ovule or pollen production in contrast to the phenotypic gender or investment that can be made into these units. In practice, components of the latter are much easier to measure.

In the present study, I examined spatial and temporal investment made by plants of *Thymelaea hirsuta* into polleniferous and ovuliferous flowers over the course of a reproductive season.

### Floral Sex Ratio

Individuals of *T. hirsuta* either produced flowers of only one sex for the length of the reproductive season (pure males or pure females), or they produced some combination of male, female and/or hermaphrodite flowers. The total number of male and female flowers produced by 305 flowering plants did not differ significantly ( $\chi^2 = 323$ ,  $n = 305$ ,  $p > 0.05$ ), indicating that at the population level, there was an equal investment into maternal and paternal resources. However, each individual plant tended to be either more male- or more female- biased in its flower production.

The 93 individuals that were not 'pure' in their sex expression produced some combination of male and female flower types. The floral sex ratio on these plants was not 1:1, but differed significantly from equality ( $t^2 = 2.00$ ,  $df = 92$ ,  $p < 0.05$ ). Moreover, this tendency is also demonstrated

investigated, providing insight into various aspects of their quantitative gender. In three species of Apiaceae, *Smyrnium olusatrum*, *Pastinaca sativa*, and *Anthriscus sylvestris*, a stable floral sex ratio of four male to one hermaphrodite flower was observed, despite a large range in the total number of flowers produced (Lovett Doust 1980). In *Aesculus pavia*, bisexual to total flower ratios ranged from 0.007 to 0.177 (Bertin 1982). O'Brien (1994) examined the variation in proportion of male flower production within a flowering season for andromonoecious *Leptospermum myrsinoides* and *L. continentale*. In a situation she called 'labile andromonoecy', the proportion of male flowers consistently increased over time, though at any one time the proportion varied widely (O'Brien 1994).

To examine temporal fluctuations in population sex ratio of *Aralia hispida*, Thomson and Barrett (1981) recorded daily counts of flowers noting if their sexual phase was male or female. Reproductive efficiency (i.e., actual maternal and paternal success) varied drastically, depending upon how many female flowers were available for pollination and how many male flowers were available to pollinate on a given day. It was assumed by Thomson and Barrett (1981) that the minority sex (in terms of flower number) will be at an advantage due to frequency-dependent selection pressures. *T. hirsuta* has a long flowering period in the field (Sept. to April, El-Keblawy 1987) and this is comparable to what was observed in the greenhouse (July to Feb.), although the peak flowering period occurred from Sept. to Dec. Lloyd's (1979, 1980) equivalence factor (total number of female flowers produced / total number of male flowers produced) has the potential to vary greatly within a season, changing functional gender estimates greatly. As well, in the field, other factors such as differential population sex ratios according to site, pollinator behaviour and nearest-neighbor distances might also affect which plant has the best potential for successful pollination with whom, and when (Willson and Burley 1983). In

reality, it is difficult to assess how closely reproductive investment and success are related.

### **Gender Distribution**

Lloyd and Bawa (1984) stressed the importance of examining the population gender distribution. They defined four main patterns which can be distinguished: 1) simultaneous cosexuality, where the distribution is unimodal and any individual plant combines male and female function in some respect; 2) gender dimorphism, where the gender distribution is bimodal and individual plants consistently specialize as males or females; 3) diphasic cosexuality where the gender distribution is bimodal but only because each individual has the potential to invest in being a maternal parent more so than a paternal parent or vice versa, in response to their current situation and; 4) phasic dimorphism where "two genetic morphs are predisposed but not irrevocably committed toward male and female modes, respectively, and in certain conditions, they are induced to switch to the other mode." (Bawa and Lloyd 1984, pg. 262).

The shape of the gender distribution of *T. hirsuta* is strongly bimodal, especially when only intermediate plants are considered (Table 2.2), despite the fact that the gender value for all plants was 0.50. This shape further emphasizes the tendency for individual plants to be largely male or female despite equal investment into maternal and paternal potential at the population level. *Thymelaea hirsuta* is not a simultaneous cosexual. However, it is difficult to distinguish conclusively among any of Lloyd and Bawa's other designations.

Allison (1991) studied gender dimorphism in *Taxus canadensis*. Although four of five populations studied were significantly bimodal, evidence that individual plants were diphasic (e.g., due to environmental or age-related factors) was deemed inconclusive (Allison 1991). In contrast,

gender was not dimorphic in *Aralia hispida* but revealed "a continuous range" as opposed to "discontinuous variation between essentially male- and female- functioning ramet" (Thomson and Barrett 1981, pg. 1102).

Without following plant gender of particular individuals from year to year, it is difficult to gain enough information to categorize accurately their sex habit. The gender distribution (standardized phenotypic femaleness) of *Gurania* and *Psiguria* species within any year is bimodal (Condon and Gilbert 1988). However, only after a combination of longterm field study following individual plants, and greenhouse observations, did it become apparent that each plant had the potential to contribute to the next generation through both maternal or paternal function. At any point in time, vines were largely unisexual but could change sex depending on their size or the time of season (Condon and Gilbert 1988).

Tan (1980) labelled the sex habit of *T. hirsuta* as trioecious, based upon herbarium specimens. This assessment failed to provide insight into the variability of gender in space and time. The present study was extremely detailed, and all flowers were counted throughout the duration of the reproductive session. This type of data lends itself to being quantitatively analyzed and is representative of plant gender continuously, for the year. However, due to the labour involved, this approach would prove to be a very difficult task for plants in the field. El-Keblawy et al. (1995) examined field populations of *T. hirsuta* and calculated gender by "formulating a visual estimate of the percentage of the different types of flowers on each plant (male, female and/or hermaphrodite)" (pg. 56), and determining relative plant femaleness (proportion of female flowers + 0.5 \* proportion of hermaphrodite flowers). Individual plants (n = 650) were followed for 3 to 6 years and 181 (28%) were stable females, producing exclusively female flowers at all observations while only 48 (7.4%) were stable males. The remaining 421 (65%) demonstrated the potential to succeed as parents through either male and/or female function at some point over the course of

the study (see Table II. in El-Keblawy et al. 1995). However, only 53 (8.2%) individuals were 'ambisexual' and demonstrated wildly fluctuating gender phenotypes within or between years (and thus inconsistent with any of the other 10 defined sex phenotypes, Chapter 1 - Table 1.2). Dommée et al. (1990, 1995) worked with French and Spanish populations of *T. hirsuta* and didn't detect any sex changes among years. Within years, they reported male (subandroecious), female (subgynoecious), protandrous and protogynous morphs. However, direct comparison with their work is difficult because it is unclear how much variability was permitted in their definitions of gender (Dommée et al. 1990, 1995).

### **Sex Choice**

The question of sex choice or gender diphasy in plants (Freeman et al. 1980, Lloyd and Bawa 1984, Schlessman 1988) and whether it exists in *Thymelaea hirsuta* is difficult to answer. Only a few examples of true gender diphasy have been demonstrated, among which are *Arisaema triphyllum* (Lovett Doust and Cavers 1982), *Catsetum viridiflavum* (Zimmerman 1991), *Panax trifolium* (Schlessman 1990), *Gurania* and *Psiguria* spp. (Condon and Gilbert 1988) and *Atriplex canescens* (Freeman et al. 1984). The main cues for changing sex are related to environmental conditions. In *Arisaema triphyllum*, for example, Lovett Doust and Cavers (1982) found that plants changed sex based upon prevailing environmental conditions. In more shaded sites with higher pH and richer nutrient soil, females were favoured compared to less shaded sites. In *Catsetum viridiflaven*, light intensity determines gender and females predominate where canopies are open (Zimmerman 1991).

Since sex choice has been found to be correlated with environmental conditions, evidence for the potential presence of gender diphasy would be the existence of spatial segregation of the sexes. Furthermore, the ability of

a plant to change its gender according to its present situation is necessary for sex choice. Gender lability among years does exist in *Thymelaea hirsuta*, although there has been no direct evidence that these gender changes are adaptive. The extent of this sex expression variability is also difficult to assess in quantitative terms, though the majority of plants remained consistent categorically (El-Keblawy et al. 1995). True phase changes, or the lack thereof can best be determined quantitatively (Schlessman 1988). Nevertheless, existence of spatial segregation of the sexes would provide some basis to conclude the presence of sex choice in *T. hirsuta*.

### **Spatial Segregation of the Sexes**

Lloyd and Webb (1977) reviewed the phenomenon of niche differences in angiosperms and found several examples of greater female prevalence at wetter sites (e.g., *Myrica gale*, *Petasites hybridus*, and *Diospyros melanoxylon*). As well, Bierzychudek and Eckhart (1988) in their review found a number of examples of this same tendency for females to be more numerous at wetter sites (e.g., *Acer negundo*, *Ephedra viridis*, *Hesperochloa kingii*, and *Salix arctica*). If sex expression can be influenced environmentally, then spatial segregation of the sexes may be an evolved response, and sex choice could be the mechanism for this response. However, SSS may also be due to a nonadaptive mechanism, such as differential mortality between the sexes (Bierzychudek and Eckhart 1988). The main reason for sexual separation, however, most likely rests upon sexual allocation theory. Females are generally thought to require more resources due to their subsequent investment into the production of fruit and seed. Males are thus 'cheaper' to produce and do not suffer as much as females in 'harsher' or more stressful environments. If greater reproductive efficiency is achieved through microhabitat separation, then spatial segregation of the sexes should be favoured.

The desert habitat provides a moisture-limiting environment for plant

species. It is possible that all sexual morphs do not experience this stress equally. Freeman and McArthur (1982) examined the water stress of twigs in six dioecious species exhibiting partial habitat segregation. The authors found that when male and female plants were compared, females suffered significantly more water stress than males in all cases. Spatial segregation in desert species may be due to female requirements for more water during fruit development.

El-Keblawy et al. (1995) discussed the notion of sexual niche separation as it applied to *T. hirsuta* and suggested that the observed male bias at dryer sites was caused by a propensity for male plants to survive at more stressful locales. However, in the greenhouse, neither gender, as calculated by the proportion of plant femaleness, nor the reproductive effort, as calculated by the total number of flowers produced, the number of male flowers produced and the number of female flowers produced, nor sex lability, were significantly affected by water treatment (Pillai's trace = 0.054,  $p = 0.31$ ). In dryer water treatment, I expected plants to produce relatively more male flowers than female flowers, based upon the habitat segregation hypothesis. However, no differences among gender were found.

Gender value estimates only the degree of femaleness within a plant irrespective of the number of flowers produced, but resource level may also affect the magnitude of reproductive effort, and act upon the absolute number of flowers produced. Male reproductive effort was measured by the number of male flowers produced and female reproductive effort by the number of female flowers produced. Total reproductive effort was measured by the total number of flowers produced by a plant over the course of the reproductive season. There were no significant effects of water treatment on reproductive effort (Table 2.3).

There are probably two main reasons why water treatment was not found to have an effect on the greenhouse-raised plants in this study in terms of their gender or reproductive effort. The first is that these variables

are relatively robust and not severely affected by moisture levels. The bias El-Keblawy et al. (1995) found in field populations might have been correlated to overall site dryness but not caused by aridity *per se*. The more southern sites in Egypt where male-biased sex ratios were found, also have different salinity levels than northern sites and also different soil types (El-Keblawy 1987). Thus, gender or reproductive effort in *T. hirsuta* may be influenced by environmental conditions correlated with, but other than moisture.

Alternatively, it is possible that the levels of watering in the greenhouse were not realistic enough to mimic harsh field conditions. The greenhouse experiment might not have elicited a response because the irrigation treatments were unlike circumstances experienced in the field. This is a common problem with studies carried out in controlled conditions. Field studies have too many conditions for one to be certain that any particular correlated variable is actually causing an observed difference. However, in greenhouse situations, one can argue that conditions are not realistic, despite the fact that one particular variable can be exclusively manipulated to test its effect. Had significant differences among water treatments been found, I could say that moisture levels influence a particular variable. By not finding an effect of water treatment, I can only say for certain that the level of irrigation I applied did not have an effect on measurements of gender in greenhouse populations of *T. hirsuta* but it leaves the effect of water on gender inconclusive. Thus from this experiment, there is no evidence of adaptive spatial segregation of the sexes in *T. hirsuta*, and thus, no evidence of sex choice.

The number of days until first flowering was significantly affected by a water-treatment-by-site-of-origin interaction. However, the differences are not amenable to simple interpretation. Site of origin alone didn't have any effect on gender, phenological or reproductive effort variables measured. Thus, I did not find any significant differences in the variables measured

among the inland, depression and coastal plants that carryover and persist in progeny.

### **Field vs. Greenhouse**

When within-reproductive season field and greenhouse data were directly compared, it was evident that a greater proportion of field plants were labile (not stable male, stable female or dichogamous) compared to greenhouse plant from the same site of origin for all sites (Table 2.5 a, b). There is a difference between the natural environment and plants grown in the greenhouse. This effect has resulted in a different qualitative sex phenotype distribution among the two, greenhouse and field *T. hirsuta* populations. Field populations are generally stable from year to year (Table XI. in El-Keblawy et al. 1995) within a site. Thus, the difference among greenhouse and field sites is not due to an inherently high degree of variability in sex phenotypes among years. However, a major difference among field and greenhouse populations is age. Greenhouse plants of *T. hirsuta* were in all cases, three years old, while the age of field populations was not determined, though presumably, most are much older than three years.

### **Maternal Sex Effects**

Dommée et al. (1995) examined the sex phenotypes of progeny of open-pollinated, protandrous, protogynous, male (subandroecious), and female (subgynoecious) morphs. They concluded that results obtained concerning gender phenotypes "confirms that it [sexual polymorphism] has a genetic basis and is not due to plant age, plant size, or environmental variation" (Dommée et al. 1995, pg. 736). Although I did not observe strictly four gender phenotypes, that these plants were grown under uniform environmental conditions (effects of water treatment were not detected) and displayed such strong gender bimodality suggests a genetic component to

the distribution observed.

From this experiment, maternal gender phenotype in *T. hirsuta* does not reflect progeny sex expression (Table 2.6). This result could reflect the variability and influence of paternal gender phenotype since progeny were obtained from open-pollinated maternal plants. Similarly, Dommée et al. (1995) found each sexual phenotype able to produce all four sexual morphs. However, they did suggest that either selection or cytoplasmic factors were biasing progeny sex ratios (Dommée et al. 1995). El-Keblawy et al. (1996b) examined maternal gender phenotype effects on *T. hirsuta* progeny and found no effect on seed mass, germination, emergency, survival after 1 month, survival after 1 year and height after 1 year. Thus, the overall effects of maternal gender phenotype on various progeny parameters seem minimal.

## **Chapter 3**

### **Self-fertility Study**

#### **Objective**

**My objective in the present investigation was to determine the effect upon fitness due to selfing in the sexually variable species, *Thymelaea hirsuta*. Additionally, I examined possible mechanisms behind any decrease in fitness with selfing. Due to the variability of sex habit in *T. hirsuta*, the presence of simultaneous functional male and female stages in some plants could permit geitonogamous (self) pollinations. Dichogamy is relatively weak in greenhouse grown plants and thus, there is potentially substantial opportunity for geitonogamy to occur. I also compared the effects of geitonogamous, half-sib and outcrossed pollinations on progeny fitness. Thus, three main questions were addressed in this study: 1) Does selfing in *T. hirsuta* result in reduced fitness? 2) If so, is the mechanism prezygotic, postzygotic or both? and 3) Does increased relatedness among parents increase any detrimental effects of selfing (suggesting the presence of inbreeding depression)?**

## **Materials and Methods**

I maintained a population of approximately 450 *Thymelaea hirsuta* plants in the greenhouse at the University of Windsor. All individuals were planted in April 1992. Some plants had reached sexual maturity (i.e., produced flowers) by 1994, but for others the 1995 reproductive season was the first time they flowered. Hand pollinations were carried out during 1995. I performed four pollination treatments: 1) outcrossed; 2) geitonogamous (self); 3) half-sib (where the pollen donor's maternal plant was the same as the pollen recipient's) and; 4) apomictic control.

For each cross to a female flower, I removed a dehiscent anther from the appropriate category of plant, using a pair of fine tweezers. Dehiscent anthers were readily detected by their bright orange, powdery appearance. Receptive female flowers could be identified by their creamy white, slightly moist stigmatic surface. Anthers were gently dabbed on the stigmatic surface. This action resulted in a transfer of orange coloured pollen grains onto the stigma. The petals of that particular flower were marked and the inflorescence tagged with an ID number. The inflorescence was then bagged with small bags of parafilm to prevent contamination. All apomictic (control) treatment flowers were female and they were bagged without any pollination.

Bags were removed after four days to prevent buildup of condensation, and reduce the chances of infection by bacteria or fungi. However receptivity of the stigma was lost shortly after pollination (typically within 48 to 72 hours - personal observation) so there was no additional risk of pollen contamination after the bags were removed. Up to five half-sib pollinations, six self pollinations, and eight outcrossed pollination were applied to each plant. Table 3.1 shows the number of pollinations performed on each plant.

A small number of hermaphrodite flowers from one plant were also

pollinated with cross pollen, or were bagged and left to self. This small sample size was due to the difficulty in identifying hermaphrodite flowers while still receptive, as well as to the rarity of this flower type (see Chapter 2). Furthermore, emasculation of hermaphrodite flowers prior to dehiscence proved difficult in practice. Thus, the outcross pollen treatment for hermaphrodites was carried out by adding additional pollen from male flowers on unrelated plants to the stigma. It could be described more accurately as a self + cross (mixed) pollination treatment.

After a month, each cross was scored as fruit set, or no fruit set. For fruits that matured, the seed was collected by peeling off the dry fruit coat. Individual seed masses were determined using a Sartorius microbalance. Germination was scored following pretreatment to optimize germination. Pretreatment was with 95% H<sub>2</sub>SO<sub>4</sub> for 10 min. prior to a 24 h treatment with 500 ppm gibberellic acid (Shaltout and El-Shourbagy 1989). Seeds were placed in individual petri dishes on wet filter paper and subjected to a 24 h dark growth chamber at 19°C. They were observed every day for two weeks, and then every two days for a further two weeks. Ungerminated seeds were tested for viability with a tetrazolium assay (Moore 1971).

### **Statistical Analyses**

Thirty-six plants were used in this pollination experiment. In an attempt to minimize among plant variance, plants were regarded as blocks within the experimental design, with five flowers randomly chosen on a plant for each pollination treatment (two for apomictic treatments). For a successful cross, both pollen donor (male flowers) and pollen recipient (female flowers) of the appropriate plants had to be in a mature stage of dehiscence or receptivity simultaneously. Missing cells in this design were the result of variability in the number and type of flowers produced by a given plant (personal observation and see El-Keblawy et al. 1995) as well as the variable phenology of flowering in a population. The numbers and type

of pollinations I carried out are shown in Table 3.1. A total of 337 crosses were carried out, using 36 plants.

All dependent variables were first tested for correlations using the Pearson correlation coefficient. Redundant variables were removed from the analysis and univariate ANOVAs were conducted separately with remaining dependent variables and cross type as a fixed independent variable. Probabilities underwent Bonferroni adjustment for simultaneous analyses. The ANOVAs were conducted to determine if cross type had a significant effect on each dependent variable employed. Analyses were carried out using plant as the unit of replication and then using flower as the unit of replication. Since *Thymelaea hirsuta* only produces one seed per fruit, fruit set was treated as equivalent to seed set, in this case.

## **Results**

**A total of 137 outcross pollinations were carried out on 34 different plants, 92 half-sib pollinations on 24 different plants, and 55 geitonogamous pollinations on 15 plants (Table 3.1). The vast majority of ovule donor plants used in this experiment were from the same water treatment (3 days). As well, since an attempt was made at a block design and to perform all four pollination treatments on the same individual, the effect of water treatment and site were sufficiently controlled for. Furthermore, plants were chosen only on the basis of flowering and having the appropriate pollen donors available (with dehiscent pollen) at the appropriate time.**

**Five "apomictic" flowers set fruit. However, it was apparent that these results reflected the activity of yellow jacket wasp contamination in the greenhouse and appropriate precautions were undertaken to assure that no contaminated flowers were included in the study. I observed these wasps moving specifically from flower to flower. After landing, they would extend their proboscis into the corolla tube for some seconds before leaving. Dehiscent pollen could easily adhere to parts of their body and become dislodged on subsequent flowers. Bagging flowers prevented contamination, as long as the stigma had not already been pollinated. However, pollinated flowers were readily detected by the layer of pollen over the stigma. Thus, as long as the stigma was carefully scrutinized for pollen and flowers bagged, there was little likelihood of contamination. As an added precaution, pollinations were not used in the study unless performed while the greenhouse was pollinator free.**

**A single plant was used to test for self-incompatibility in hermaphrodite flowers. Five flowers were simply bagged, without any manipulations (selfed treatment). Orange pollen from surrounding anthers were clearly visible on the stigma of these hermaphrodite flowers. Five others were emasculated and bagged (control), although it is likely that some**

self pollen had already reached the stigma. I treated just two flowers with additional cross pollen and then proceeded to bag them. None of these pollinations resulted in fruit set.

Fruit set was recorded in two different ways. At the level of the flowers, fruit set occurred, or did not occur (1 or 0). However, at the level of the plant, the proportion of flowers that set fruit for a particular pollination treatment was determined (number of flowers that set fruit / number of flowers pollinated). The seed mass for each flower setting fruit was determined. As well, on a per plant basis, the mean seed mass per pollination treatment was determined (though the variance of individual seed masses was lost). Germination for each seed was scored as a dichotomous variable, based on whether germination occurred (radicle emergence) or did not. On a per plant basis, the percentage of germinated seeds was determined (seeds that germinated from a particular pollination treatment for a particular plant / seeds pollinated for that treatment). Tetrazolium assays determined that only 9 of the seeds that failed to germinate were still viable. Thus the vast majority of nongerminating seeds were nonviable.

Tabachnick and Fidell (1989) have argued against the use of several correlated dependent variables in separate ANOVAs, as the  $\alpha$  value increases at an unknown rate. However, if variables are uncorrelated, then separate ANOVAs may be performed, so long as the  $\alpha$  level for each test is adjusted to maintain an overall significance level of 0.05. There are three dependent variables used in this study: fruit set, seed mass and germinability. Tests for correlation among these variables revealed that seed weight and germinability were highly significantly correlated (Pearson correlation coefficient = 0.682,  $p < 0.001$ ). Thus, to reduce the redundancy of variables, and bypass the problems of correlation among dependent variables, germinability was eliminated from further analyses. Seed weight and fruit set were uncorrelated (Pearson correlations coefficient = 0.054,  $p = 1.00$ ). Separate ANOVAs were performed using a Bonferroni-adjusted

significance level of 0.025 to maintain an overall probability value of 0.05.

Using the plant as the unit of replication, a one-way ANOVA with pollination treatment as the independent variable was performed (Table 3.2). To better conform to assumptions of normality, proportion fruit set was arcsine transformed. There was no significant effect of pollination type on proportion fruit set ( $F = 0.76$ ,  $df = 2,70$ ,  $p = 0.47$ ). The mean proportion fruit set ( $\pm$  SE) of each cross type is shown in Figure 3.1. As well, there was no significant effect of cross type on mean seed mass ( $F = 1.90$ ,  $df = 2,47$ ,  $p = 0.16$ ) (see Table 3.3). Figure 3.2 shows the mean seed mass per plant ( $\pm$  SE) for each cross type. However, the selfed treatment produced the lowest fruit set, making seeds with a lower seed mass than either of the other pollination types.

A power analysis can provide information about the minimum sample size required to detect a significant difference for any particular variable. It should then be possible to answer whether or not the result obtained is nonsignificant due to an actual lack of differences among pollination treatments or because of insufficient power. According to Zar (1984), the sample size required,  $n$ , can be determined by the following formula:

$$n = (t_{0.05}^2 \cdot s^2) / (D^2 \cdot Y^2)$$

where  $t_{0.05}$  is the  $t$  value at a significance level of 0.05,  $D$  is the desired precision (typically set at 0.20),  $s^2$  is the sample variance and  $Y$  is the sample mean. For fruit set,  $n = 95$  and for seed mass, the value  $n = 104$ . Thus, because a sample size of 73 ( $n_{\text{self}} = 15$ ,  $n_{\text{half-sib}} = 24$ ,  $n_{\text{outcross}} = 34$ ) was employed, it is possible that there may have been a difference among pollination treatments but that the sample size simply wasn't sufficient to detect it.

Generally, flowers should not be used as the unit of replication because flowers on a plant are not necessarily independent units. However, because flowers on a plant in this experiment were chosen randomly in terms of their spatial and temporal distribution, it can be argued that flowers

within a plant are no more or less correlated than flowers among plants. On this premise, it is possible to treat each flower as an independent unit. Separate ANOVAs with fruit set and seed mass as dependent variables were performed (Table 3.4, 3.5).

Fruit set (Fig. 3.3) did not differ significantly among pollination treatments ( $F = 2.97$ ,  $df = 22, 280$ ,  $p = 0.053$ ). However, it is apparent that values approached significance. Seed mass (Fig. 3.4) was significantly different among pollination treatments ( $F = 4.06$ ,  $df = 2, 115$ ,  $p = 0.020$ ). Furthermore, a Tukey HSD post hoc test revealed that the significant difference lay between seed mass of geitonogamous and half sib crosses ( $p < 0.020$ ). The difference between geitonogamous and outcross pollinations was not significant ( $p = 0.072$ ).

Self-incompatibility can be quantified by determining the index of self-incompatibility (ISI). The ISI is calculated by taking the ratio between fruit set resulting from self-pollination to fruit set resulting from outcross pollination (Bullock 1985). A value above 0.25 indicates that the species is self-incompatible (Bawa 1974, Bullock 1985). ISI for *Thymelaea hirsuta* was found to be 0.65 and thus is in effect, partially self-incompatible.

The effects of inbreeding can be quantified by using the inbreeding depression coefficient,  $\delta$  (sigma). This value is equal to  $1 - w_i/w_o$  where  $w_i$  is the fitness of the selfed treatment and  $w_o$  is the fitness of the outcrossed treatment (see also Johnston 1992, O'Neil 1994). As inbreeding depression can only be manifest postzygotically,  $\delta$  was estimated for seed mass and germination using mean values.  $\delta = 0.23$  for seed mass and 0.35 for germinability.

**Table 3.1. Number of pollinations of each cross type, performed on flowering plants. The plant ID indicates the maternal plant. Apomictic treatments were only bagged, half-sib pollinations were performed using pollen donors with the same mother as pollen recipients, selfed treatments were geitonogamous and outcrossed pollinations were between flowers from unrelated plants.**

| <b>Plant ID</b> | <b>Apomictic</b> | <b>Half-Sib</b> | <b>Selfed</b> | <b>Outcrossed</b> | <b>Total</b> |
|-----------------|------------------|-----------------|---------------|-------------------|--------------|
| 8.1             | 1                | 0               | 0             | 7                 | 8            |
| 79.1            | 0                | 2               | 0             | 1                 | 3            |
| 98.1            | 2                | 3               | 0             | 3                 | 8            |
| 107.2           | 2                | 3               | 3             | 3                 | 11           |
| 116.1           | 2                | 5               | 0             | 5                 | 12           |
| 118.2           | 1                | 2               | 0             | 4                 | 7            |
| 121.2           | 2                | 3               | 0             | 3                 | 8            |
| 128.1           | 1                | 1               | 0             | 0                 | 2            |
| 146.1           | 0                | 0               | 2             | 1                 | 3            |
| 154.1           | 2                | 5               | 0             | 5                 | 12           |
| 154.2           | 0                | 0               | 3             | 2                 | 5            |
| 159.3           | 2                | 3               | 0             | 3                 | 8            |
| 166.2           | 2                | 4               | 0             | 5                 | 11           |
| 171.1           | 2                | 4               | 4             | 4                 | 14           |
| 172.2           | 1                | 0               | 5             | 3                 | 9            |
| 173.3           | 2                | 5               | 0             | 5                 | 12           |
| 179.1           | 1                | 3               | 3             | 3                 | 10           |
| 179.2           | 0                | 3               | 0             | 3                 | 6            |
| 179.3           | 2                | 5               | 0             | 5                 | 12           |
| 220.2           | 2                | 0               | 0             | 6                 | 8            |
| 223.2           | 1                | 2               | 0             | 2                 | 5            |
| 235.1           | 2                | 0               | 3             | 5                 | 10           |
| 236.1           | 1                | 0               | 2             | 3                 | 6            |
| 245.1           | 1                | 0               | 4             | 3                 | 8            |
| 263.3           | 2                | 0               | 0             | 5                 | 7            |
| 274.3           | 2                | 4               | 0             | 5                 | 11           |
| 295.1           | 2                | 4               | 0             | 4                 | 10           |

**Table 3.1. continued**

|              |           |           |           |            |            |
|--------------|-----------|-----------|-----------|------------|------------|
| <b>306.3</b> | <b>1</b>  | <b>4</b>  | <b>6</b>  | <b>5</b>   | <b>16</b>  |
| <b>315.1</b> | <b>2</b>  | <b>5</b>  | <b>0</b>  | <b>8</b>   | <b>15</b>  |
| <b>315.2</b> | <b>2</b>  | <b>5</b>  | <b>0</b>  | <b>5</b>   | <b>12</b>  |
| <b>319.3</b> | <b>1</b>  | <b>4</b>  | <b>4</b>  | <b>4</b>   | <b>13</b>  |
| <b>320.3</b> | <b>2</b>  | <b>3</b>  | <b>3</b>  | <b>3</b>   | <b>11</b>  |
| <b>322.1</b> | <b>2</b>  | <b>5</b>  | <b>5</b>  | <b>5</b>   | <b>17</b>  |
| <b>322.2</b> | <b>3</b>  | <b>0</b>  | <b>5</b>  | <b>4</b>   | <b>12</b>  |
| <b>384.3</b> | <b>0</b>  | <b>0</b>  | <b>3</b>  | <b>0</b>   | <b>3</b>   |
| <b>385.1</b> | <b>2</b>  | <b>5</b>  | <b>0</b>  | <b>5</b>   | <b>12</b>  |
| <b>Total</b> | <b>53</b> | <b>92</b> | <b>55</b> | <b>137</b> | <b>337</b> |

**Table 3.2 Results of ANOVA for effect of pollination type on proportion fruit set (arcsine transformed) using plant as the unit of replication. Significance was tested at the level,  $p = 0.025$ , due to Bonferroni-adjustment for simultaneous analyses.**

| Source     | SS     | df | MS    | F ratio | p     |
|------------|--------|----|-------|---------|-------|
| Cross Type | 0.463  | 2  | 0.231 | 0.757   | 0.473 |
| Error      | 21.386 | 70 | 0.306 |         |       |

**Table 3.3 Results of ANOVA for effect of pollination type on seed mass using plant as the unit of replication. Significance was tested at the level,  $p = 0.025$ , due to Bonferroni-adjustment for simultaneous analyses.**

| Source     | SS     | df | MS    | F ratio | p     |
|------------|--------|----|-------|---------|-------|
| Cross Type | 3.515  | 2  | 1.757 | 1.898   | 0.161 |
| Error      | 43.512 | 47 | 0.926 |         |       |

**Table 3.4 Results of ANOVA for effect of pollination type on fruit set using flower as the unit of replication. Significance was tested at the level,  $p = 0.025$ , due to Bonferroni-adjustment for simultaneous analyses.**

| Source     | SS     | df  | MS    | F ratio | p     |
|------------|--------|-----|-------|---------|-------|
| Cross Type | 1.447  | 2   | 0.723 | 2.969   | 0.053 |
| Error      | 68.221 | 280 | 0.244 |         |       |

**Table 3.5 Results of ANOVA for effect of pollination type on seed mass using flower as the unit of replication. Significance was tested at the level,  $p = 0.025$ , due to Bonferroni-adjustment for simultaneous analyses.**

| <b>Source</b>     | <b>SS</b>      | <b>df</b>  | <b>MS</b>    | <b>F ratio</b> | <b>p</b>     |
|-------------------|----------------|------------|--------------|----------------|--------------|
| <b>Cross Type</b> | <b>9.742</b>   | <b>2</b>   | <b>4.871</b> | <b>4.055</b>   | <b>0.020</b> |
| <b>Error</b>      | <b>138.153</b> | <b>115</b> | <b>1.201</b> |                |              |

### **Legend to Figures in Chapter 3**

**Figure 3.1. Mean proportionate fruit set ( $\pm$ SE) per plant for each pollination treatment ( $n_{\text{self}} = 15$ ,  $n_{\text{half-sib}} = 24$ ,  $n_{\text{outcross}} = 34$ ).**

**Figure 3.2. Mean seed mass ( $\pm$ SE) per plant for each pollination treatment ( $n_{\text{self}} = 9$ ,  $n_{\text{half-sib}} = 15$ ,  $n_{\text{outcross}} = 26$ ).**

**Figure 3.3. Mean proportionate fruit set ( $\pm$ SE) per flowers for each pollination treatment ( $n_{\text{self}} = 55$ ,  $n_{\text{half-sib}} = 92$ ,  $n_{\text{outcross}} = 136$ ).**

**Figure 3.4. Mean seed mass ( $\pm$ SE) per flower for each pollination treatment ( $n_{\text{self}} = 16$ ,  $n_{\text{half-sib}} = 34$ ,  $n_{\text{outcross}} = 68$ ).**

**Figure 3.5. Mean proportionate seed germination ( $\pm$ SE) for each pollination treatment.**

Figure 3.1

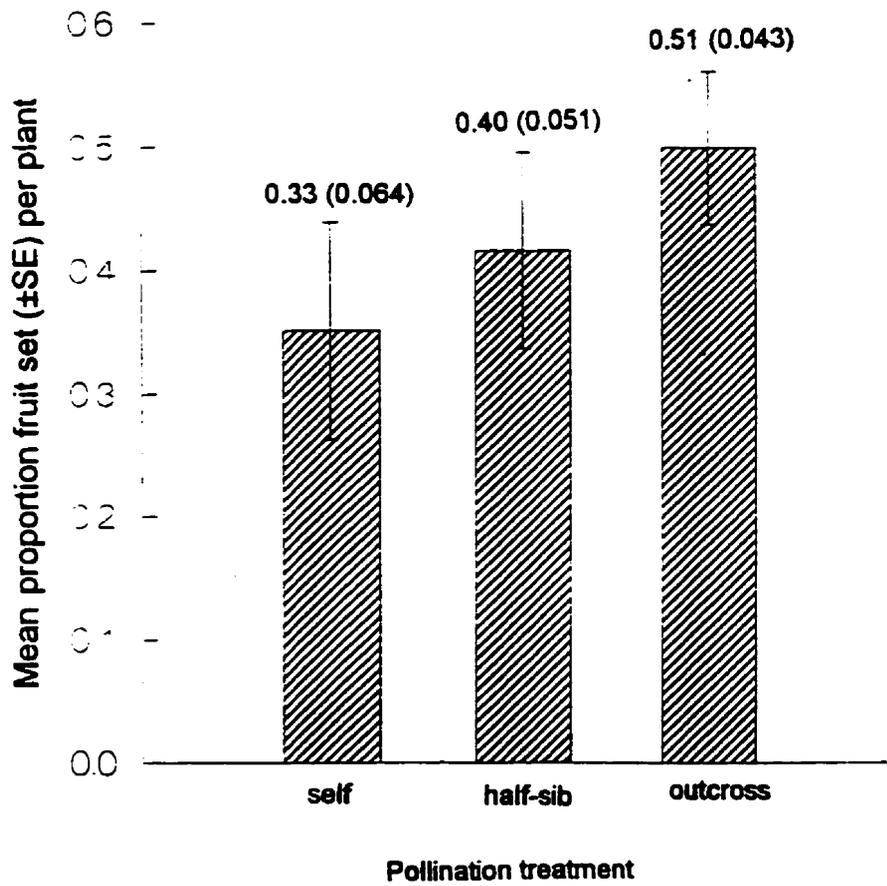


Figure 3.2

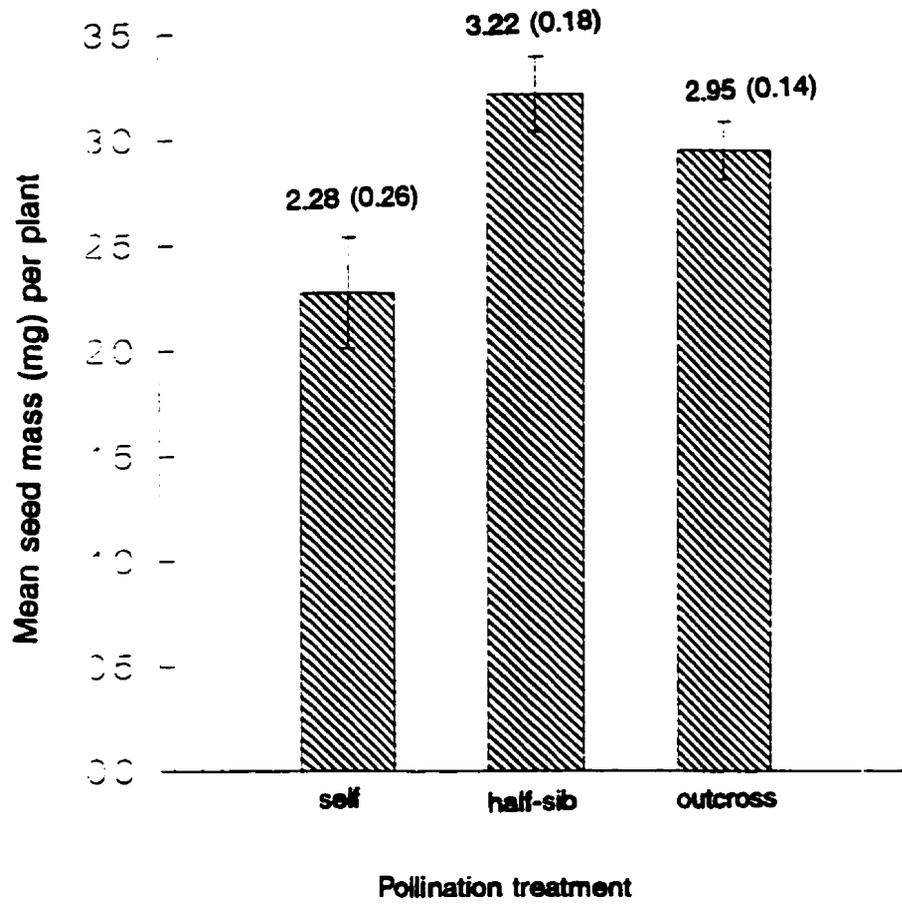


Figure 3.3

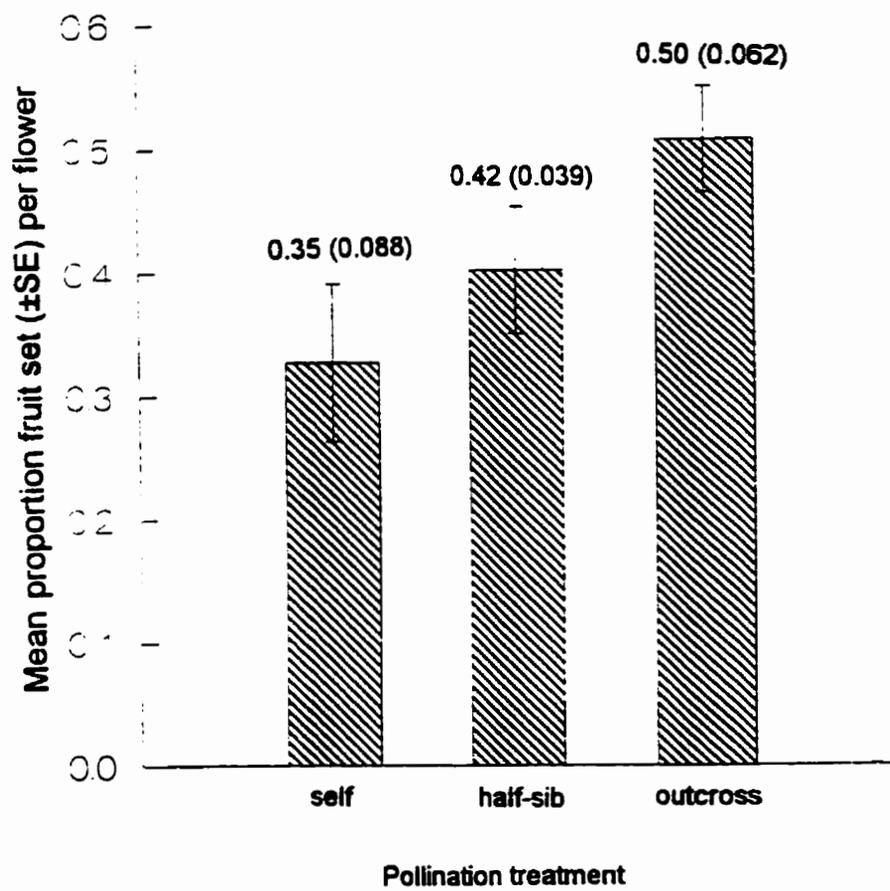


Figure 3.4

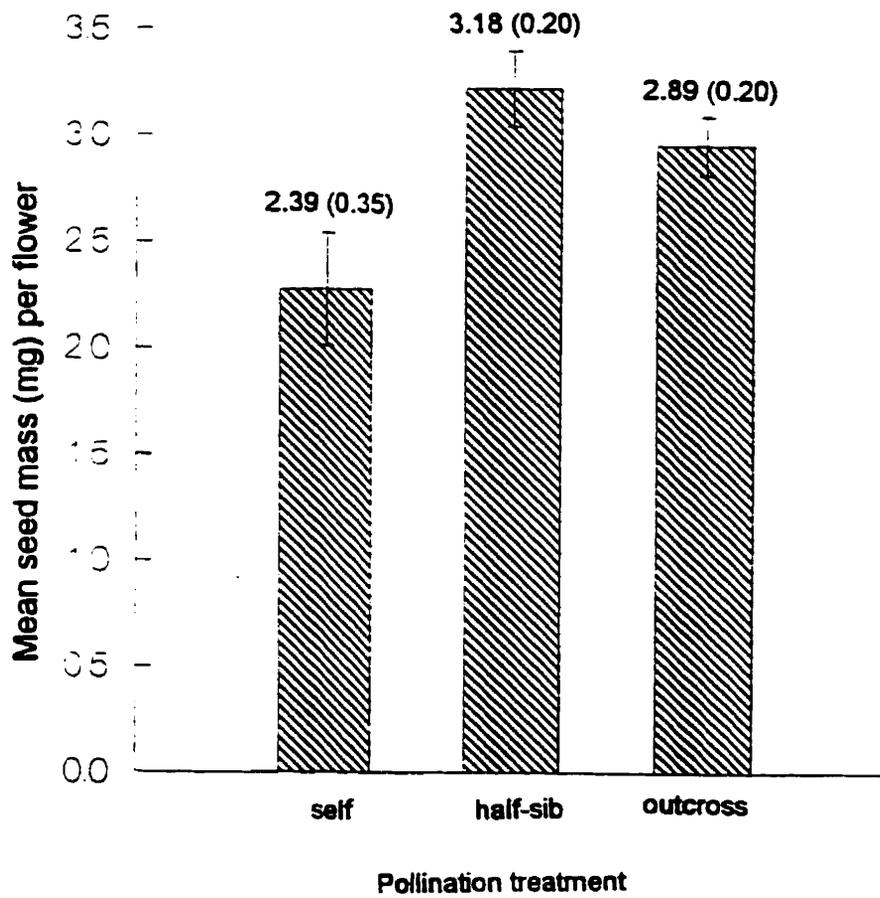
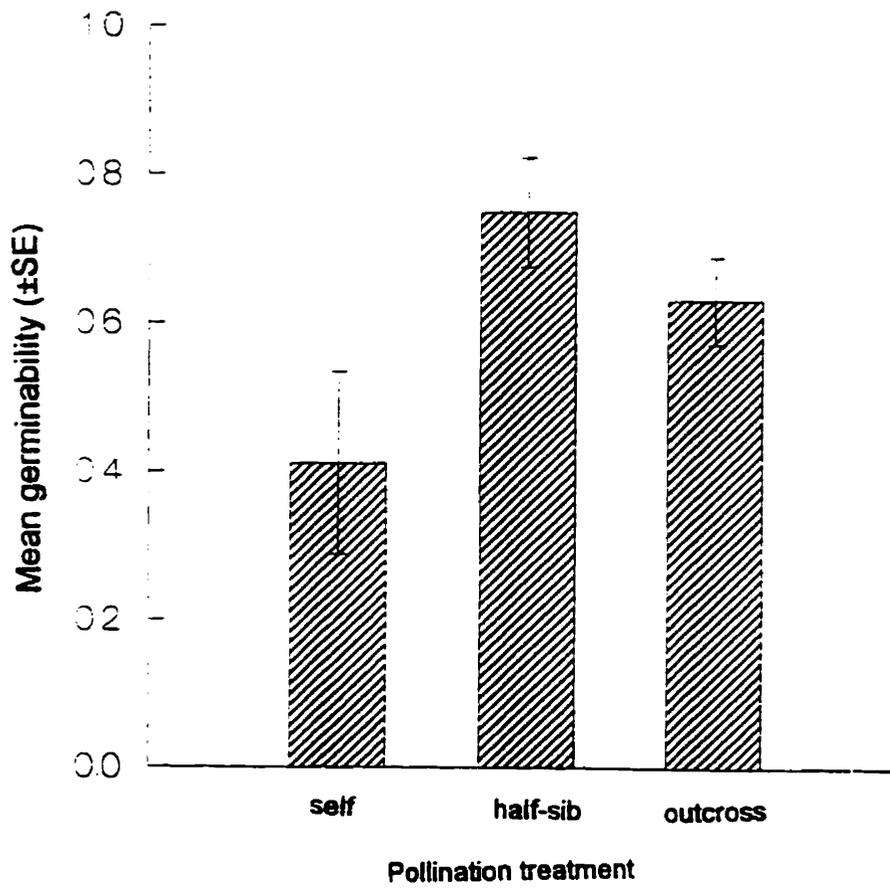


Figure 3.5



## Discussion

### Selfing Effects

Selfing has been shown to reduce fitness in a number of plant species. These include several *Vochysia* species (Oliveira and Gibbs 1994), *Epilobium obcordatum* (Seavey and Carter 1994), members of the *Asclepiadaceae* (Wyatt and Broyles 1994), *Lupinus* spp. (Karoly 1994), *Thymus vulgaris* (Assouad et al. 1978), *Sagittaria* spp. (Delesalle and Muenchow 1992), *Lythrum salicaria* (O'Neil 1994), *Persoonia mollis* (Krauss 1994), *Geranium caespitosum* (Hessing 1988), *Lobelia cardinalis* and *L. siphilitica* (Johnston 1992), *Epilobium angustifolium* (Parker et al. 1995) and *Dianthus chinensis* (Aizen et al. 1990). Selfing can occur through either self-pollination of hermaphrodite flowers or geitonogamy, the pollination of female flowers by males on the same individual. Although there were very few hermaphrodite flowers to be found in our greenhouse population of *Thymelaea hirsuta* (see Chapter 2), approximately one third of all plants produce male and female flowers, often simultaneously (Fig. 2.1, Table 2.1). Thus there exists a strong potential for geitonogamy to occur in *T. hirsuta*. Selfing in *T. hirsuta* resulted in reduced progeny fitness, compared to either outcrossed or half-sib pollination treatments. For all measures of fitness, the selfed pollination treatment resulted in lower values (Fig. 3.3, Fig. 3.4). This result was significant for seed mass but not significant for fruit set.

The mechanism for the detrimental effects of selfing is not always apparent. There are three main ways in which selfing can reduce fitness: through self-incompatibility systems, inbreeding depression and seed abortion (see Barrett 1988, Wyatt and Broyles 1994). Assouad et al. (1978) noted that the average number of seeds produced by male fertiles (hermaphrodites) of *Thymus vulgaris* that were selfed was significantly lower than outcrossed male fertiles. They stated that this result "can be

selfed progeny is inbreeding depression. Inbreeding depression is widespread (Seavey and Carter 1994, Karoly 1994, Hessing 1988, Parker et al. 1995, Montalvo 1992, Johnston 1992) although fitness is measured in various currencies. Hessing (1988) found that although geitonogamously pollinated flowers initiated the same number of fruits as outcrossed ones, the mean seed mass was significantly lower. However, Johnston (1992) found no difference in seed mass between outcrossed and selfed progeny but did detect inbreeding depression in the later stages of growth. In *T. hirsuta*, there was a significant difference among pollination treatments for seed mass ( $F = 4.06$ ,  $df = 2, 280$ ,  $p = 0.020$ ), suggesting that inbreeding depression plays a role in the reduction of selfed progeny fitness (Fig. 3.4).

The coefficient of inbreeding depression,  $\delta$ , was found to be 0.23 for seed mass and 0.35 for seed germinability. This index of inbreeding depression varies continuously from -1 to 1, where values tending to 1 would indicate severe inbreeding depression. Values around 0 would imply no differences between the performance (fitness) of selfed and outcrossed products, and negative values would imply that selfing has a beneficial effect on the resulting progeny over outcrossed treatments. Johnston (1992) determined inbreeding depression values for various stages in the life cycle of *L. cardinalis* and *L. siphilitica*. Using survival as a measure of fitness, from seed to germination, and from germination to six weeks, the cumulative inbreeding depression coefficient ranged from 0.10 to 0.46. Inbreeding depression calculated by using mean seed mass in *Lythrum salicaria* was between -0.09 and -0.25 indicating that there were no negative effects of inbreeding (O'Neil 1994). However, subsequent measures of  $\delta$ , calculated with variables such as germination rate, number of leaves at 10 days, length of longest leaf at harvest and final dry mass, were positive and ranged up to 0.64. Thus, there is a potential for inbreeding effects at subsequent stages in the *T. hirsuta* life cycle, which were not examined.

The results of inbreeding depression on plants maintained in natural

field environments have been compared with those grown greenhouse environments. Dudash (1990) suggested that inbreeding depression is greater in more harsh environments (but see Delesalle and Muenchow 1992). Johnston (1992) found inbreeding depression lower for greenhouse plants than field populations of *Lobelia cardinalis*. Seavey and Carter (1994) report that field collected plants had a mean seed set of 5% when selfed while greenhouse progeny had a mean seed set of 11% when selfed.

Thus, due to the fact that inbreeding depression in *T. hirsuta* was only measured employing a short portion of the progeny life cycle, there is a potential for present inbreeding depression estimates to be very conservative. Selfing produces a reduced seed set through both prezygotic and postzygotic mechanisms (although prezygotic effects were not significant).

### **Maternal Choice**

There are potentially two main postzygotic effects, inbreeding depression or maternal choice acting through ovule abortion. Theoretically, they are readily distinguished, since inbreeding depression is a function of an individual's particular genotype while ovule abortion is determined by the maternal plant producing overall higher quality offspring. Thus, inbreeding depression can act through various stages of a plant's lifecycle while ovule abortion occurs shortly after embryo formation. However, it is difficult to discern the mechanism if the effects of selfing also occur shortly after zygote formation. The effects of maternal choice through ovule abortion and early-acting inbreeding depression are difficult to de-couple. This problem occurs whether there is one seed per fruit or many multisired seeds. If seed weight is low, or germination fails, there is a confounding effect of inbreeding depression and maternal choice.

Maternal 'choice' to abort an ovule can be due to the position of the ovule in the fruit and competition for limited resources (Rigney 1995).

However, the maternal plant may also abort an ovule due to inferior genetic quality or mutational load (Rigney 1995). In this latter case, ovule abortion is both a function of the progeny genotype as well as the maternal plant's 'decision' to allow ovule abortion (Willson and Burley 1983).

If inbreeding depression was the only factor in affecting seed mass and germinability then we would expect that as parental relatedness increased, the more severe inbreeding depression would be, due to an increased likelihood of accumulating deleterious recessive alleles. However, when we examine the trend in seed mass and germinability, it is apparent that the results of half-sib pollinations were significantly greater than geitonogamous pollinations, but not significantly different than outcross pollinations and actually a tendency to slightly greater mean seed mass, or germinability occurs (Fig. 3.4, 3.5). Thus, it is possible that additional factors such as maternal choice play a role in postzygotic effects of selfing in addition to inbreeding depression.

## **Chapter 4**

### **Architecture Study**

#### **Objective**

There were two main objectives for this part of my study. The first was to describe architectural structure in *Thymelaea hirsuta*. Plant growth form is very flexible and likely influenced by environmental variables, as well as by genetic factors. Due to the aridity associated with desert environments and the limitations of water as a resource, I hypothesized that irrigation level could have an influence on plant morphology. I asked if there were differences among irrigation treatment in plant architecture over the course of a period of one year, during which repeated observations were taken.

The second goal was to determine if there were differences among the relative placement of male, female and hermaphrodite flowers within a plant. Positional effects in plants have been shown to be based upon acquisition of resources, and proximity to resource sinks, or subject to dispersal or pollination pressures (Tomlinson 1987, Cox 1988, Waller 1988). I hypothesized that the positions of particular flower types (i.e., male, female or hermaphrodite) on a plant are not random. Sex allocation theory suggests that females require more resources, and thus would be located closer to resource sinks than male flowers. If environmental conditions affect architecture, and architecture constrains sex expression, then it should be reasonable to postulate a link between environmental circumstances and sex expression mediated through plant architecture.

## **Materials and Methods**

### **Effect of Irrigation Treatment On Plant Architecture**

In August of 1994, approximately three hundred two year old plants of *Thymelaea hirsuta* were selected from three different water treatments for architectural study. Water treatments involved differences in watering frequency. A third of the plants received 250 mL every three days, another third received 250 mL every six days and the last third, every nine days. On each of these plants, five primary branches were selected and tagged. One branch was randomly selected from the top third of the plant, three branches were taken from the middle third and the remaining branch from the bottom third. In this way, branches from all parts of the plant were represented.

The length of each primary branch was measured, along with any secondary, tertiary, and higher order branches. Thus for each plant, the total length of five primary branches could be calculated along with the total length of any secondary branches, and third order or higher order branches on each primary branch. The number of branches of each order arising from each primary branch was recorded. Height was also measured for each plant.

These measurements were repeated every two months on the same branches for 12 months. If a branch died, it was replaced with another branch in the same portion of the plant, unless none was available. In that case, another branch elsewhere on the plant was selected. In total, six repeated measurements were recorded. The last set of measurements was not used for analysis because too many branches needed to be replaced, and plant mortality was very high. If whole plants died, they were replaced with another plant from the same water treatment.

### **Statistical Analyses**

In this study, repeated measures were taken on several dependent

variables. When different individuals are sampled repeatedly, and measures taken that may be correlated to one another, the problem is doubly multivariate (Tabachnick and Fidell 1996). I used BMDP's 4v procedure to examine the effect of water treatment and time on height, total length of primary branches, total length of secondary branches, total length of third order branches, number of secondary branches and number of third order branches. Higher order branches were omitted due to their infrequent nature. However, a second similar analysis was conducted with the dependent variables: height, total branch length (the sum of five primary branches with all higher order branches) and total number of branches (the sum of the number of secondary and higher order branches).

BMDP will not accept missing values and thus only complete cases could be analyzed. Plants that died, or plants replacing dead plants were not included in these analyses. In total 202 plants were used. This type of analysis accounts for both the fact that dependent variables may be correlated, and that measurements of the same individual taken over time may be correlated (repeated measures).

### **Position Effects**

Just over 100 flowering plants of *Thymelaea hirsuta* were randomly sampled from a set of 305 raised in the greenhouse at the University of Windsor. All 305 plants were not used in the analysis due to the danger of Type II error (Tabachnick and Fidell 1989). These plants were surveyed each month for the number of male, female and hermaphrodite flowers (see Chapter 2). For each flower on these 100 plants, various positional attributes were recorded: 1) Each flower was assigned a branch order (ord) corresponding to its level of branching; 2) The total number of branches of that particular order was determined, as well as the rank of the particular branch with flowers. For example, a flower may have appeared on a secondary branch (ord = 2). That branch may have been the 5th secondary

branch (rank = 5) out of a total of 7 secondary branches produced by its 'parent' primary branch (Fig. 4.1). The rank of this 5th branch was standardized by the total number of branches of that order (thus orank =  $5 / 7$ , in this case); 3) The third positional character recorded was the rank of the primary-order branch containing the flower, regardless of which level the flower was on. Thus, in Figure 4.1, the flower is on the 4th primary branch from a total of 9 primary branches (and the variable prank =  $4 / 9$ ). Note that if flowers were directly located on the primary level, then orank = prank; 4) Height of the plant was also recorded. Ord and orank reflected the distance of a particular flower from the main stem, while prank gave an indication of the vertical dimension, how far up the plant the flower was located. Plant height provided information on the absolute size of plants on which flowers appeared.

### **Statistical Analyses**

A total of 12,488 flowers were used in the analysis, 7,282 were male, 5,162 were female and the remaining 44 were hermaphrodite. A multivariate analysis of variance was carried out to test the effect of flower sex on ord, orank, prank and height. Dependent variables were tested for correlation and a stepwise ANCOVA was performed to determine the effects of each variable after the effect of correlated measures were removed. A discriminant function analysis was also performed to determine the effect of architectural variables as predictors of flower sex.

## **Results**

### **Effects of Irrigation Treatment on Plant Architecture**

The multivariate repeated measures procedure testing the effect of water treatment and time on height, length of five primary branches, length of secondary branches, length of tertiary branches, number of secondary branches and number of tertiary branches showed an overall highly significant effect of water x time interaction (Wilks' Lambda = 0.599,  $F = 2.14$ ,  $df = 48, 352$ ,  $p < 0.0001$ ).

When the effects of each dependent variable were examined individually, water treatment, time and water treatment x time interaction were all significant for height (Table 4.1). Figure 4.2 demonstrates that as water treatment becomes less frequent (and thus "harsher"), the rate of plant growth significantly decreases. There may also be a slight effect of the length of primary branches that contributes to the overall effect of water treatment ( $F = 3.70$ ,  $df = 2, 199$ ,  $p = 0.026$ ). However, Figure 4.3 indicates that there are very small differences between the response of plants from different water treatments with respect to this measure. The other dependent variables showed no effect of water treatment, nor any interaction effects. All variables contributed to the overall significant effect of time (Table 4.1; see Fig. 4.4, 4.5, 4.6, 4.7).

By the time of the last time, the mean total length of secondary branches (58.5 cm) exceeded the mean total length of primary branches (41.3 cm). However, the mean total length of tertiary branches remained very low (7.6 cm). As well, while there were over 30 secondary branches per plant (for five primary branches), the number of tertiary branches was, on average, less than 9 per plant. Thus, an absolute increase in both branch number and length appears at the second order. However, it is apparent that the rate of growth is greater at the third order in number of branches and overall length (see Fig. 4.4, 4.5, 4.6, 4.7)

In the second analysis, branch length and branch number was summed over all branch orders. The result was consistent with the previous analysis. There was an overall highly significant effect of a water treatment x time interaction (Wilks's Lambda = 0.704,  $F = 3.02$ ,  $df = 24, 378$ ,  $p < 0.0001$ ). Height contributed to the effect of water treatment, time and the interaction (Table 4.2), as expected from Table 4.1. The total length of branches and the total number of branches were both highly significant for a time effect, but showed no effect on water treatment or interaction (Table 4.2). Figures 4.8 and 4.9 show the growth rate of branch length and number of branches respectively. The results of this analysis confirm the results of the first analysis and indicate that orders above the third did not contribute significantly to water and time effects.

#### **Position Effects**

A MANOVA examining the effect of flower sex on four position variables (branch order [ord], standardized rank of branch of that order [orank], standardized rank of primary branch [prank] and plant height) (see Materials and Methods for explanation of variables) was highly significant (Wilks' Lambda = 0.864,  $F = 236.0$ ,  $p < 0.001$ ). Clearly, the spatial arrangement of male, female and hermaphrodite flowers was not random with respect to one another. A Pearson correlation matrix revealed that all pairwise correlations were highly significant ( $p < 0.001$ ) with Bonferroni-adjusted probability levels. The exception was height and orank which were not related at all ( $p = 1.00$ ). Univariate ANOVAs conducted on each variable separately revealed that the variance contributing to the total was greatest for height, and then decreased for ord, prank and orank respectively. A stepwise ANCOVA was performed to determine which variables differed significantly for sex of flower and thus contributed to the overall significant effect.

First, a univariate ANOVA showed that height differed significantly

among flower sex ( $F = 574.0$ ,  $df = 2$ ,  $12467$ ,  $p < 0.001$ ). Fig. 4.10 demonstrates that male flowers tended to occur on taller plants (mean  $\pm$  SE =  $73.1 \pm 0.149$ ) than female flowers (mean  $\pm$  SE =  $65.3 \pm 0.180$ ), and hermaphrodite flowers tended to occur on the shortest plants (mean  $\pm$  SE =  $59.9 \pm 0.406$ ). The results of hermaphrodite flowers should be treated with caution due to the small sample size ( $n = 44$ ).

Height was then treated as the first covariate in an ANCOVA and there were significant differences found among flower sex in branch order ( $F = 176.7$ ,  $df = 2$ ,  $12466$ ,  $p < 0.001$ ). Figure 4.11 shows the mean branch order for each sex. Female flowers tended to occur on higher branch orders, further away from the main stem and primary branches than male flowers. Hermaphrodites tended to occur on the higher branch orders, compared with either male or female flowers.

With both height and branch order (ord) as covariates in an ANCOVA to test the effects of sex on prank, there was a significant result ( $F = 187.0$ ,  $df = 2$ ,  $12465$ ,  $p < 0.001$ ). Figure 4.12 shows the tendency for male flowers to be positioned higher on a plant than female flowers, and hermaphrodites to be positioned lower. There was no significant effect of flower sex on orank when the effects of height, ord and prank were removed.

A discriminant function analysis was carried out to determine how well these four descriptors could predict flower sex. The overall analysis was significant (Pearson Chi-square =  $1678.6$ ,  $df = 6$ ,  $p < 0.0001$ ). However, the predictive power placed only 55% of flowers in the correct sex categories, based solely upon positional characteristics alone. Many male and female flowers were miscategorized as hermaphrodites.

**Table 4.1. Results of repeated measures MANOVA testing the effects of water treatment and time on height, primary length, secondary length, tertiary length, number of secondary branches, and number of primary branches. The contribution of each dependent variable to the overall effect is also shown.**

| Source                   | Test Statistic | F       | df     | p       |         |
|--------------------------|----------------|---------|--------|---------|---------|
| <b>Water</b>             |                |         |        |         |         |
| Overall Effect           | Wilks' Lambda  | 0.811   | 3.58   | 12, 388 | <0.0001 |
| height                   | MS             | 12574.5 | 11.25  | 2, 199  | <0.0001 |
| primary length           | MS             | 3444.6  | 3.70   | 2, 199  | 0.026   |
| secondary length         | MS             | 21816.6 | 1.92   | 2, 199  | 0.149   |
| tertiary length          | MS             | 342.6   | 0.99   | 2, 199  | 0.373   |
| no. of secondary         | MS             | 6133.5  | 3.17   | 2, 199  | 0.044   |
| no. of tertiary branches | MS             | 415.2   | 1.14   | 2, 199  | 0.322   |
| <b>Time</b>              |                |         |        |         |         |
| Overall Effect           | Wilks' Lambda  | 0.247   | 56.66  | 24, 176 | <0.0001 |
| height                   | Hotelling's T  | 481.6   | 118.57 | 4, 196  | <0.0001 |
| primary length           | Hotelling's T  | 443.7   | 109.26 | 4, 196  | <0.0001 |
| secondary length         | Hotelling's T  | 136.8   | 33.68  | 4, 196  | <0.0001 |
| tertiary length          | Hotelling's T  | 44.6    | 10.98  | 4, 196  | <0.0001 |
| no. of secondary         | Hotelling's T  | 211.8   | 52.15  | 4, 196  | <0.0001 |
| no. of tertiary branches | Hotelling's T  | 47.9    | 11.81  | 4, 196  | <0.0001 |
| <b>Time x Water</b>      |                |         |        |         |         |
| Overall Effect           | Wilks' Lambda  | 0.599   | 2.14   | 48, 352 | 0.0001  |
| height                   | Wilks' Lambda  | 0.776   | 6.63   | 8, 392  | <0.0001 |
| primary length           | Wilks' Lambda  | 0.956   | 1.12   | 8, 392  | 0.346   |
| secondary length         | Wilks' Lambda  | 0.958   | 1.06   | 8, 392  | 0.387   |
| tertiary length          | Wilks' Lambda  | 0.983   | 0.43   | 8, 392  | 0.903   |
| no. of secondary         | Wilks' Lambda  | 0.953   | 1.20   | 8, 392  | 0.299   |
| no. of tertiary branches | Wilks' Lambda  | 0.961   | 0.98   | 8, 392  | 0.453   |

**Table 4.2. Results of repeated measures MANOVA testing the effect of water treatment and time on height, total length of five primary branches and higher orders, total number of branches on primary branches. Overall effects of water, time and time x water treatment are significant.**

| Source              | Test Statistic |         | F      | df      | p      |
|---------------------|----------------|---------|--------|---------|--------|
| <b>Water</b>        |                |         |        |         |        |
| Overall Effect      | Wilks' Lambda  | 0.842   | 5.92   | 6, 396  | <0.001 |
| height              | MS             | 12601.2 | 11.11  | 2, 200  | <0.001 |
| length              | MS             | 51555.3 | 2.42   | 2, 200  | 0.092  |
| no. of branches     | MS             | 9428.6  | 2.80   | 2, 200  | 0.063  |
| <b>Time</b>         |                |         |        |         |        |
| Overall Effect      | Hotelling's T  | 852.34  | 67.12  | 12, 189 | <0.001 |
| height              | Hotelling's T  | 529.22  | 145.83 | 4, 197  | <0.001 |
| length              | Hotelling's T  | 174.13  | 42.88  | 4, 197  | <0.001 |
| no. of branches     | Hotelling's T  | 175.81  | 43.29  | 4, 197  | <0.001 |
| <b>Time x Water</b> |                |         |        |         |        |
| Overall Effect      | Wilks' Lambda  | 0.704   | 3.02   | 24, 378 | <0.001 |
| height              | Wilks's Lambda | 0.767   | 7.00   | 8, 394  | <0.001 |
| length              | Wilks's Lambda | 0.962   | 0.95   | 8, 394  | 0.471  |
| no. of branches     | Wilks's Lambda | 0.962   | 1.13   | 8, 394  | 0.341  |

## **Legend to Figures for Chapter 4**

**Figure 4.1.** Schematic drawing of *Thymelaea hirsuta*, showing how positional measurements were taken. The variable orank = 5/7, while prank = 4/9. See text for further explanation.

**Figure 4.2.** Mean ( $\pm$ SE) plant height for each water treatment per time. Circles represent watering frequency of 3 days, squares represent watering frequency of 6 days and diamonds represent watering frequency of 9 days. Plant height increases with time but the rate of growth in each water treatment is significantly different (see Tables 4.1 and 4.2).

**Figure 4.3.** Mean ( $\pm$ SE) length of 5 primary branches for each water treatment. Circles represent watering frequency of 3 days, squares represent watering frequency of 6 days and diamonds represent watering frequency of 9 days. Length of primary branches increases over time but response for each water treatment is similar.

**Figure 4.4.** Mean ( $\pm$ SE) secondary branch length for 5 primary branches demonstrating a significant effect of time. There was no effect of water treatment (Table 4.1).

**Figure 4.5.** Mean ( $\pm$ SE) tertiary branch length for 5 primary branches per plant, showing a significant effect of time. There was no effect of water treatment (Table 4.1).

**Figure 4.6.** Mean ( $\pm$ SE) number of secondary branches on 5 primary branches of *Thymelaea hirsuta* demonstrating a significant effect of time. There was no effect of water treatment (Table 4.1).

**Figure 4.7.** Mean ( $\pm$ SE) number of tertiary branches on 5 primary branches of *Thymelaea hirsuta* demonstrating a significant effect of time. There was no effect of water treatment (Table 4.1).

**Figure 4.8.** Mean ( $\pm$ SE) length of 5 primary branches and all higher order branches over all times. The increase over time was significant but water treatment was not (Table 4.2).

**Figure 4.9.** Mean ( $\pm$ SE) number of branches of all orders on 5 primary branches in *Thymelaea hirsuta*. There was a significant effect of time but not of water treatment (Table 4.2).

**Figure 4.10.** Mean ( $\pm$ SE) height for male, female and hermaphrodite flowers.

**Figure 4.11. Mean ( $\pm$ SE) branch order for male, female and hermaphrodite flowers.**

**Figure 4.12. Mean ( $\pm$ SE) prank measure (see Materials and Methods for explanation) for male, female and hermaphrodite flowers.**

Figure 4.1

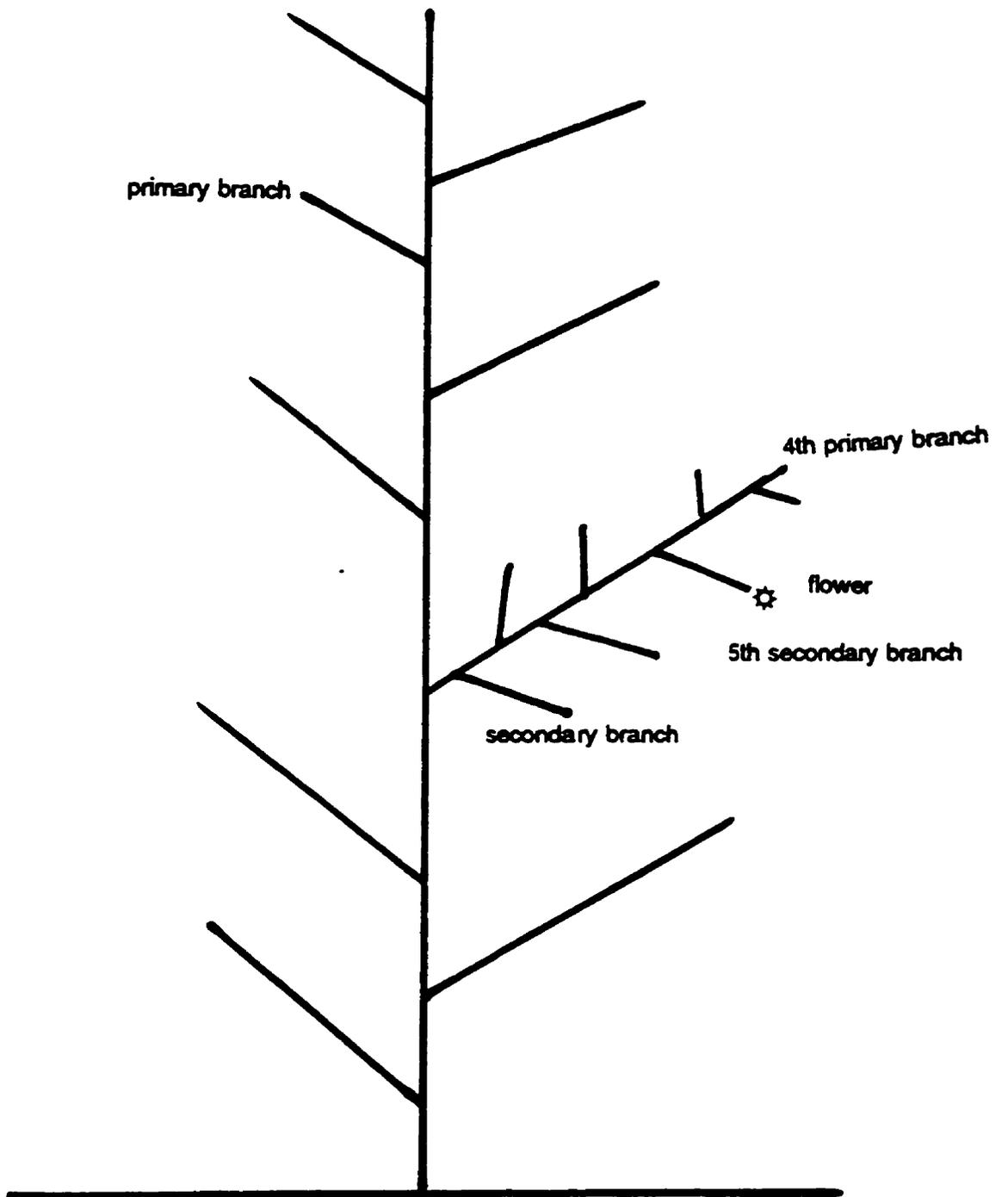


Figure 4.2

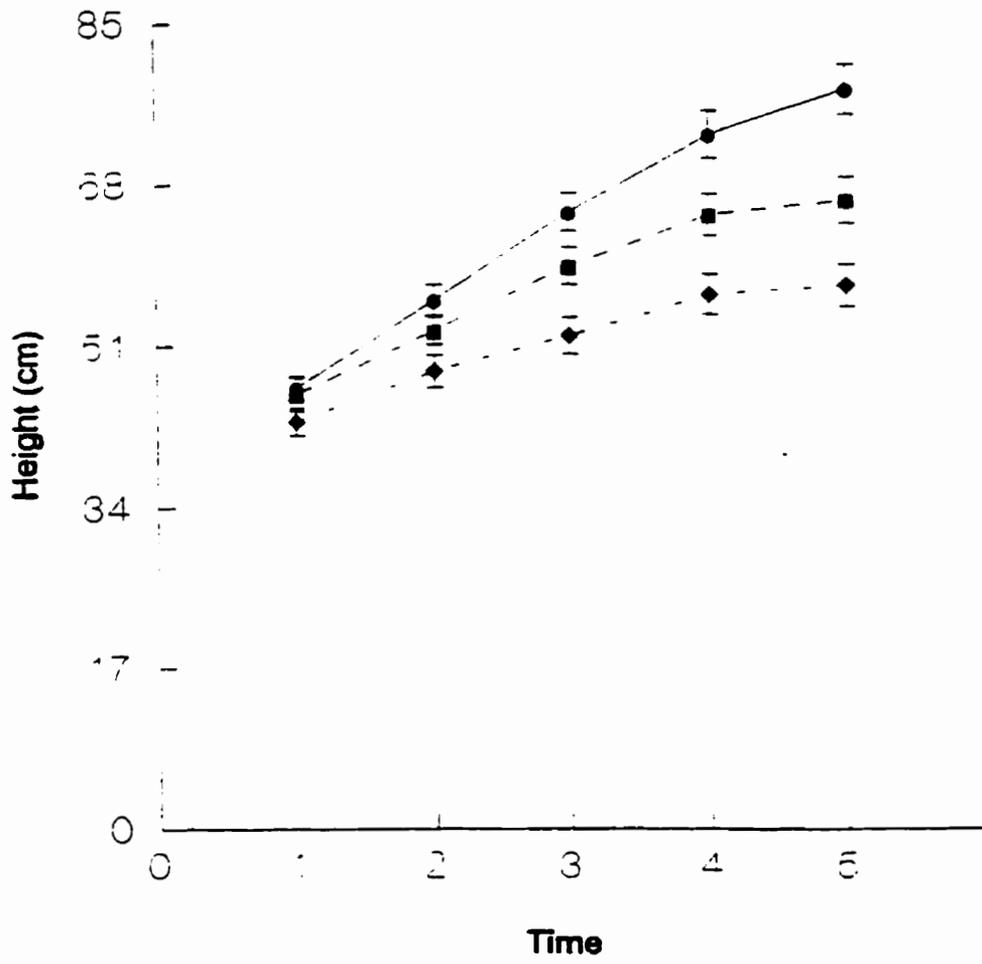


Figure 4.3

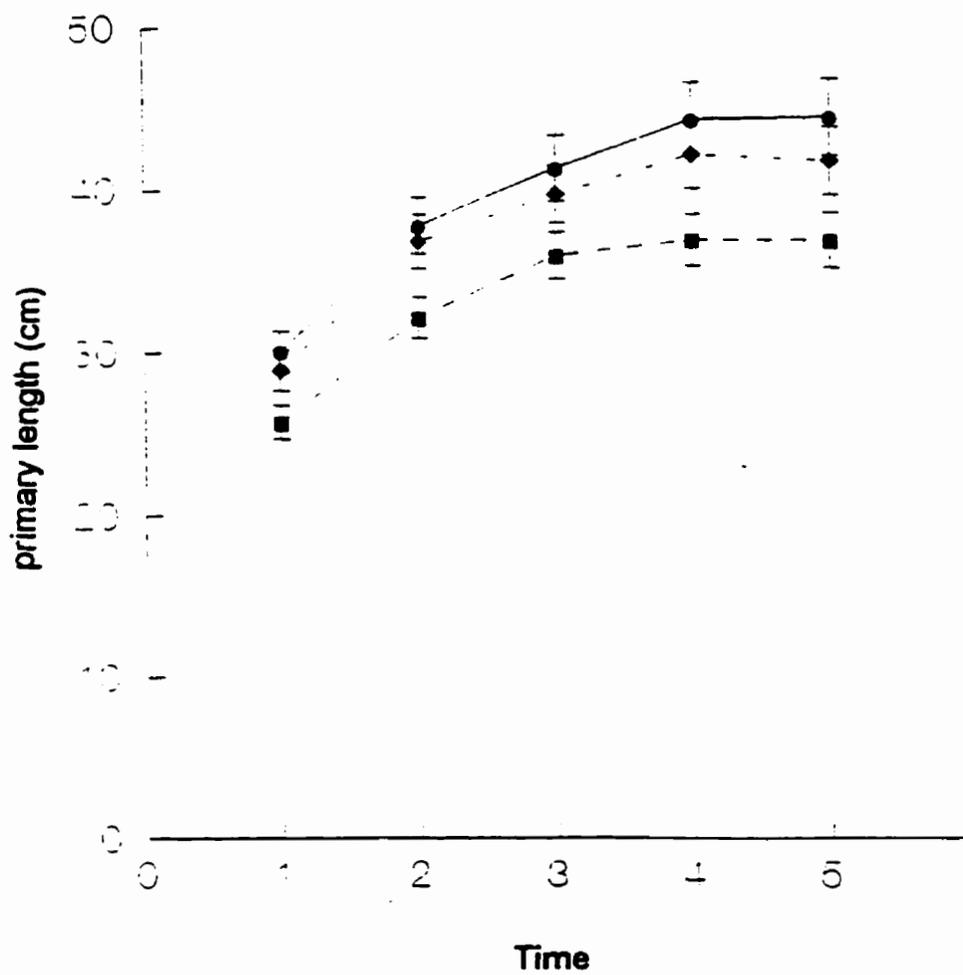


Figure 4.4

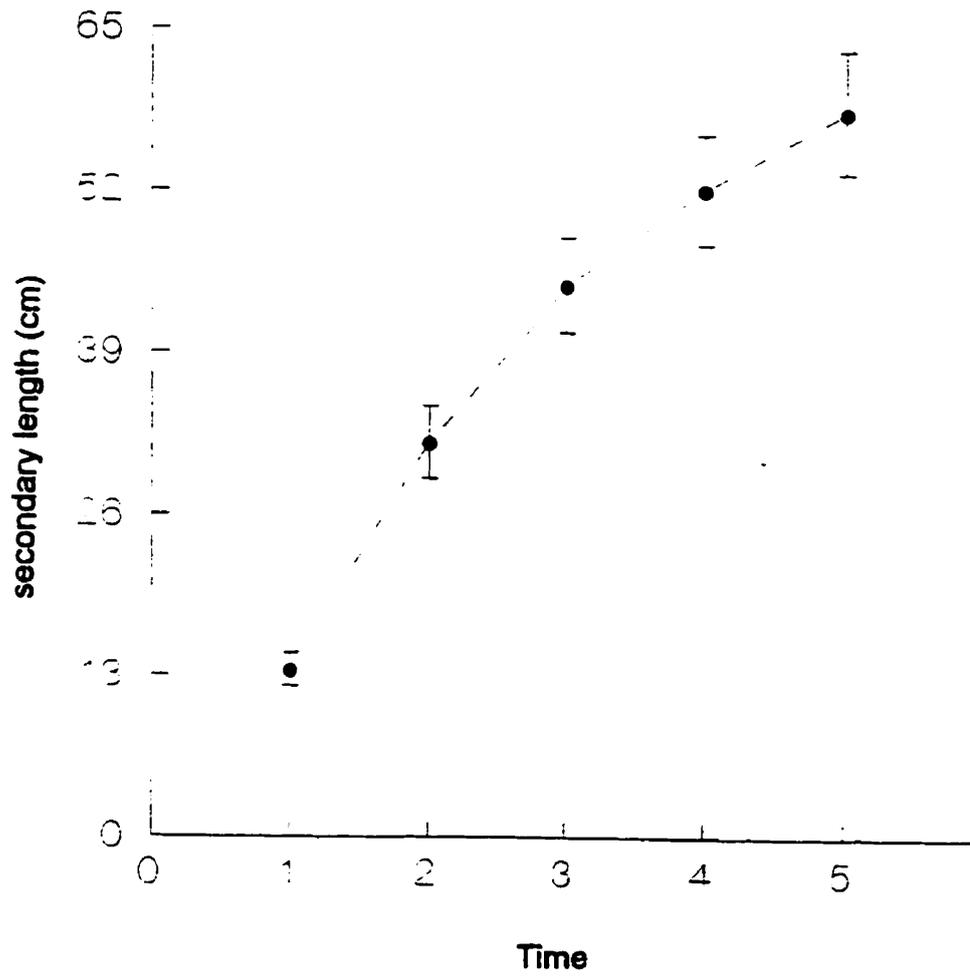


Figure 4.5

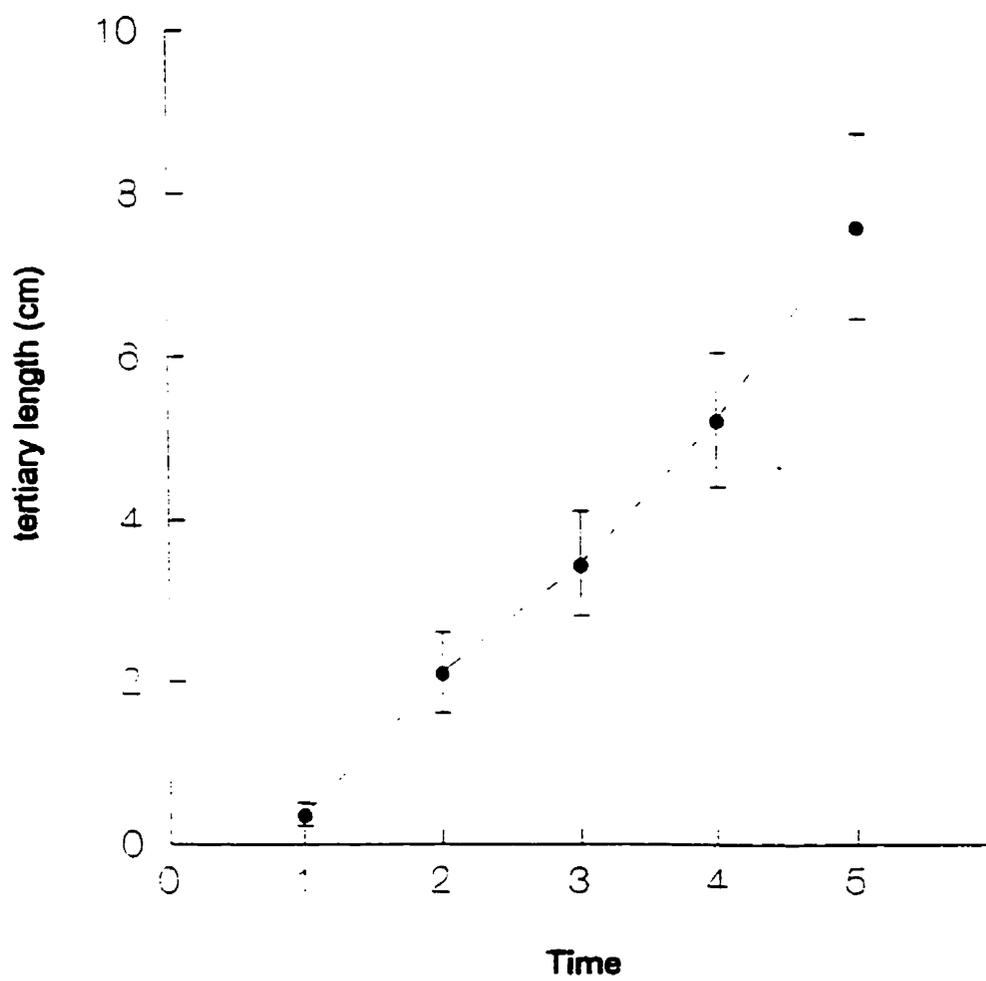


Figure 4.6

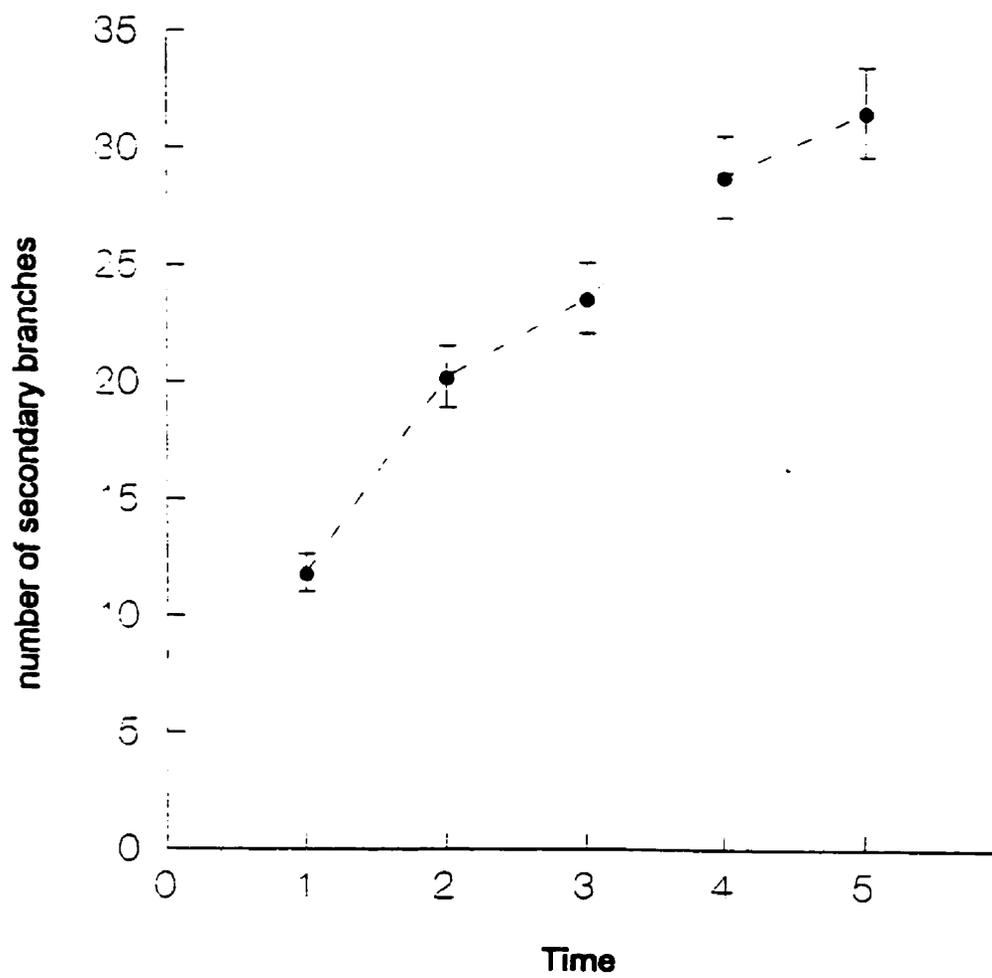


Figure 4.7

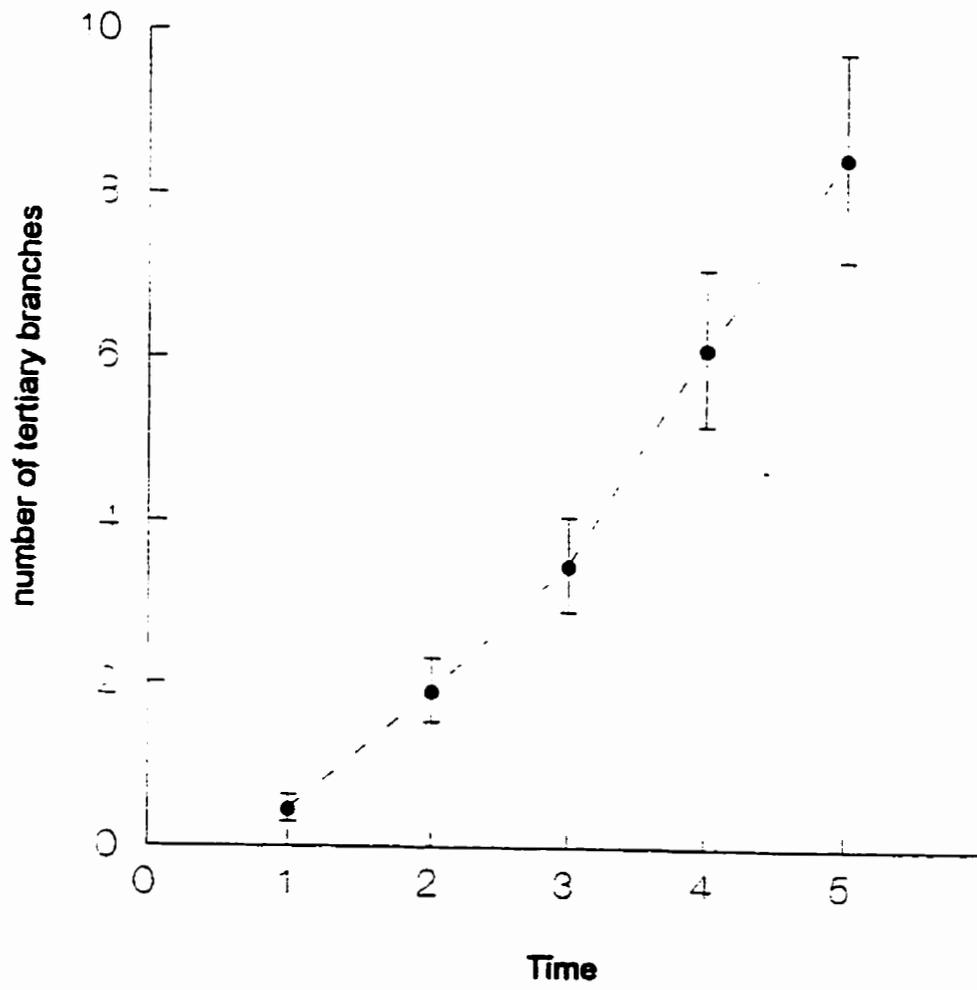


Figure 4.8

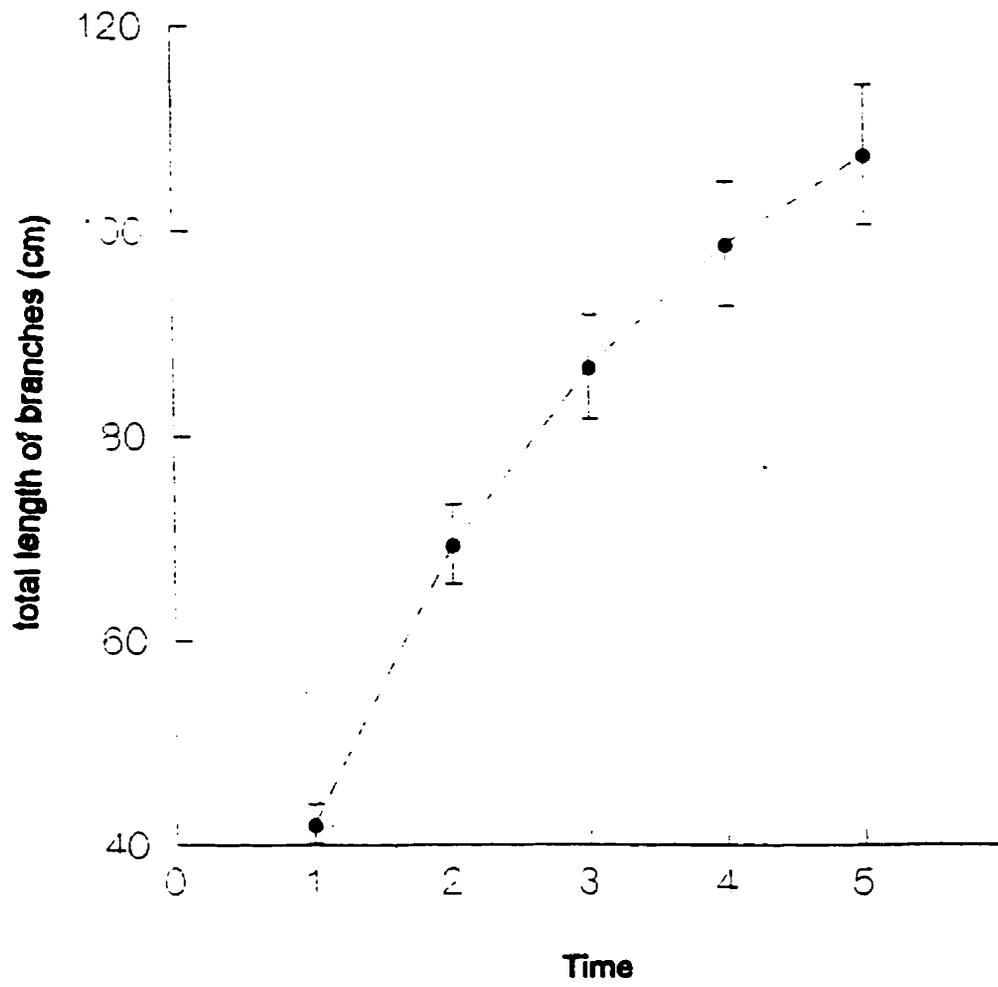


Figure 4.9

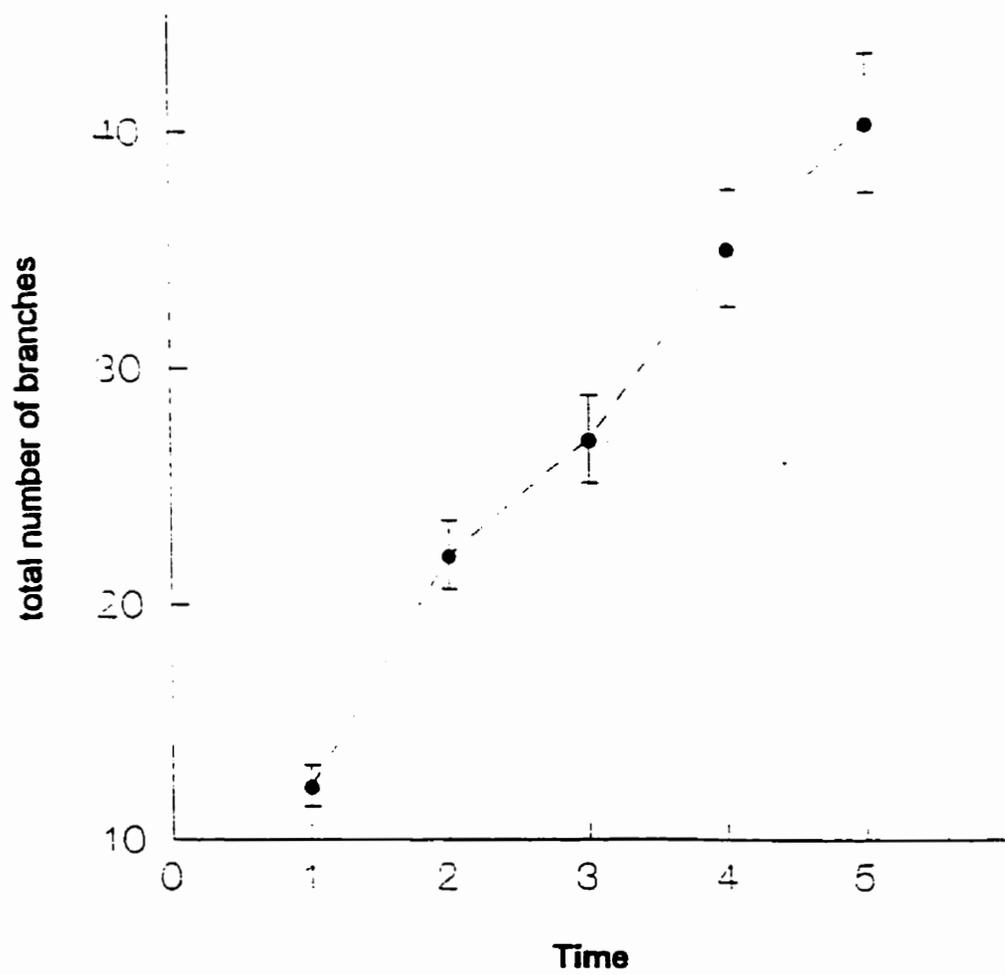


Figure 4.10

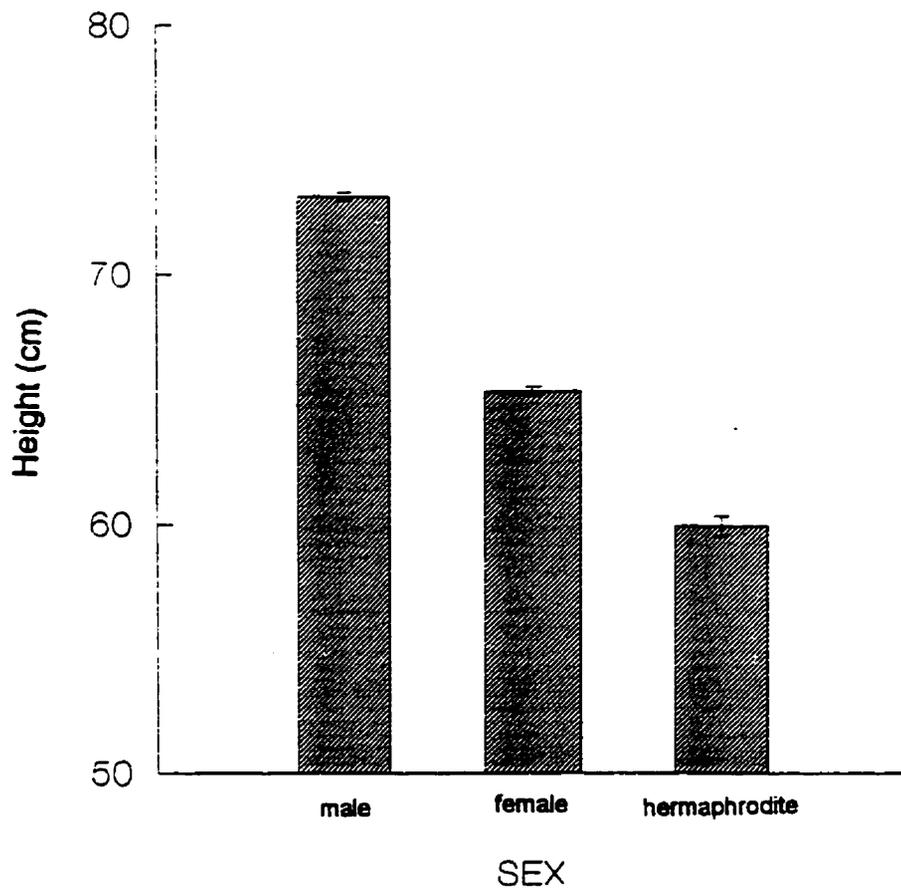


Figure 4.11

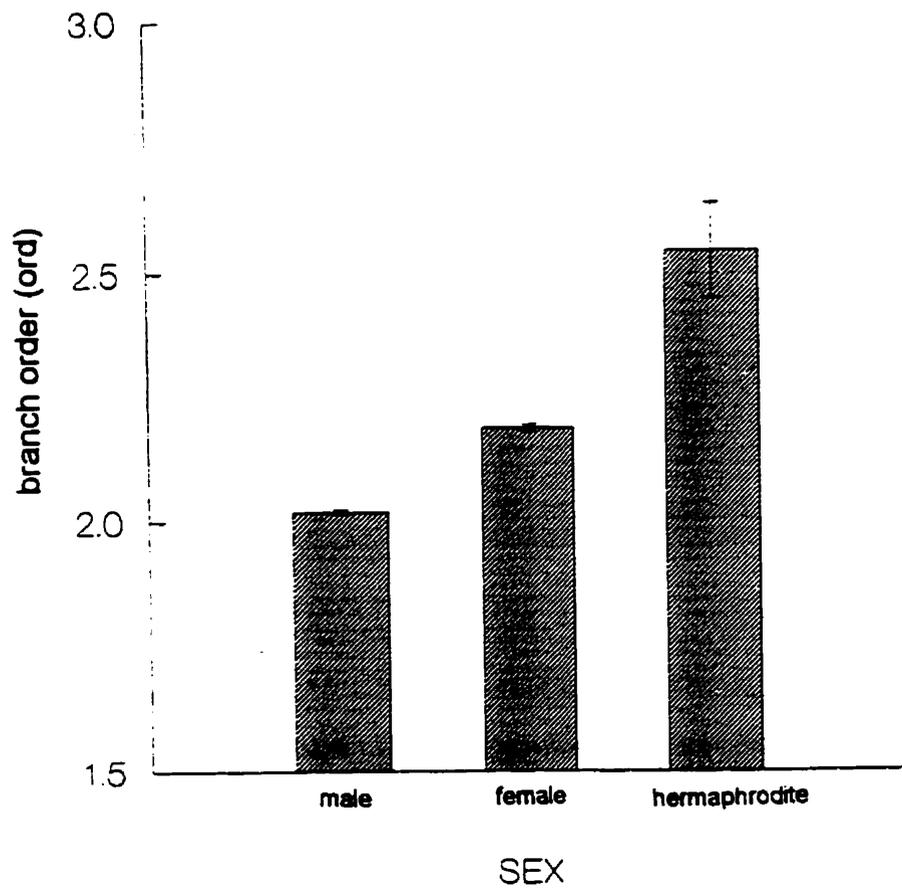
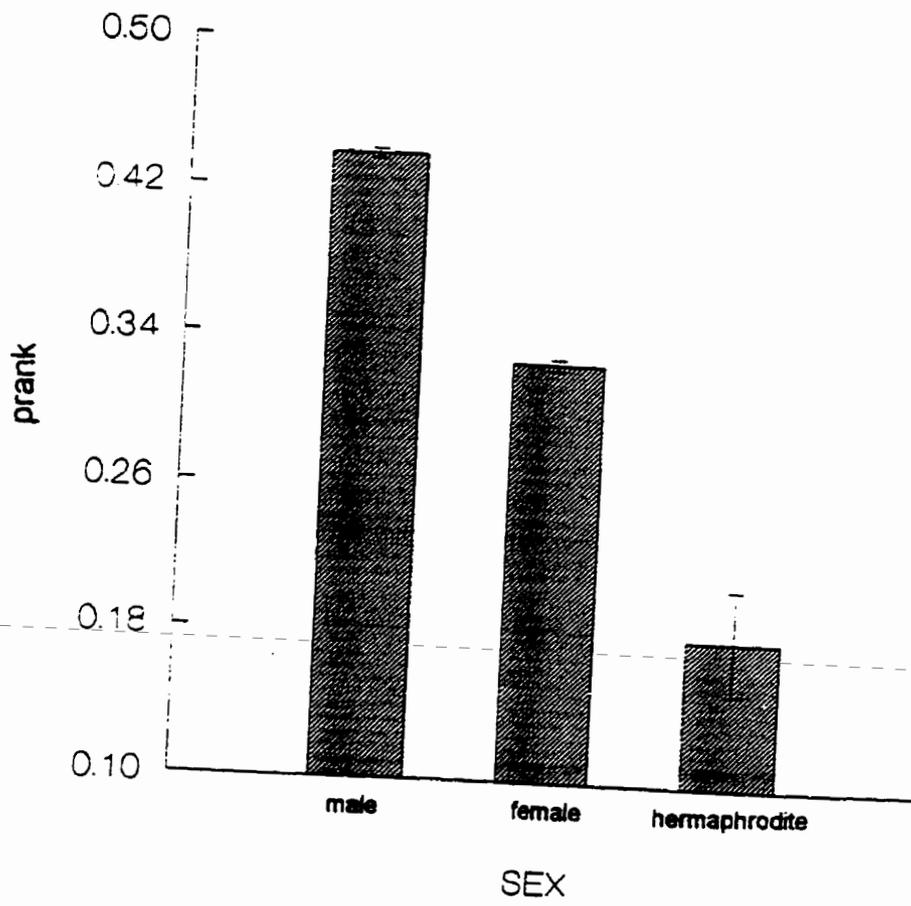


Figure 4.12



## Discussion

### Environmental Effects on Plant Architecture

The photosynthetic capacity of plants is dependent upon water availability and, thus, it was not surprising that growth in *Thymelaea hirsuta* was significantly diminished in xeric environments. Reduced carbon assimilation can be caused directly by low moisture levels, as is evident in drought conditions. In the present experiment, plants of *Thymelaea hirsuta* were subject to three different irrigation level. Plants were watered every 3 days, every 6 days or every 9 days. The significant effect of water treatment (Wilks' Lambda = 0.811,  $F = 3.58$ ,  $df = 12, 388$ ,  $p < 0.0001$ ) on plant architecture demonstrates that the irrigation levels applied were extreme enough to elicit a detectable response. As expected, plant architecture was also significantly affected by time (Wilks' Lambda = 0.247,  $F = 556.7$ ,  $p < 0.0001$ ), indicating substantial growth over the course of the 10 month study period (Fig. 4.2 - 4.6). Furthermore, the significant interaction effect (Wilks' Lambda = 0.599,  $F = 2.14$ ,  $p < 0.0001$ ) suggests that plants from the three water treatments were not homogeneous in their growth response.

All of the observed architectural parameters increased significantly over time, contributing to the overall significance of the time effect (Table 4.1, 4.2). However, with respect to the main effect of water treatment and the interaction between water treatment and time, only height contributed significantly to the overall effect (Table 4.1, 4.2). Figure 4.2 demonstrates graphically that not only does increasing water availability increase absolute plant height in *Thymelaea hirsuta*, but it also increases the rate of growth.

The modularity of plant growth allows a large degree of plasticity in plant form (Bradshaw 1965, Cox 1988, Silvertown and Lovett Doust 1993). Environmental effects have the potential to influence this architectural variability. The effect of water stress in plants is obvious to anyone who

has experienced drought conditions or neglected to water their garden. More specifically, in *Larrea tridentata*, moisture availability affects vegetative production as well as reproductive allocation (Cunningham et al. 1979). In another case, in *Laportea canadensis* soil texture (and thus water availability) significantly affects growth, (Menges 1987). In terms of height and dry weight, sandy soils were found to depress growth. However, within a certain range of moisture availability, architectural plasticity may be an adaptive response. In *Cercidium floridum*, for example, leaves are produced only after moisture availability has been assured through rainfall, and they are shed with the onset of xeric conditions (Bradshaw 1962).

More recently, there have been several studies examining the effect of light (canopy gaps) on specific components of plant architecture. Menges (1987) found that an increase in light intensity is positively correlated with height, leaf production and changes in actual leaf size and morphology in *Laportea canadensis*. Sipe and Bazzaz (1994) observed an increase in number of branches, number of leaves and total leaf area with larger canopy openings in several *Acer* species. Similarly, differences in canopy structure, as a consequence of different neighbour species affected a number of architectural parameters, such as number of nodes, stem mass and maximum leaf area in *Datura stramonium* and *Polygonum pensylvanicum* (Tremmel and Bazzaz 1995).

### **Position Effects**

In *T. hirsuta*, the position of flowers was not random. There were significant differences in position among male, female and hermaphrodite flowers (Wilks' Lambda = 0.864, F = 236.0, p < 0.001). In fact, discriminant analysis revealed that given position characteristics, the sex of a flower could be accurately predicted 55% of the time.

The actual locational differences between male and female flowers are

worth highlighting. Three main trends were observed: 1) Male flowers were produced on taller plants than female flowers, whereas hermaphrodite flowers were produced on shorter plants than female flowers (Fig. 4.10); 2) Male flowers were positioned relatively higher on plants than female flowers, but female flowers were positioned higher than hermaphrodites (Fig. 4.12) and; 3) Female flowers were more likely to be found on higher-order branches than male flowers, but less likely to be found on lower-order branches than hermaphrodite flowers (Fig. 4.11).

Since plants of *Thymelaea hirsuta* demonstrate a continuous distribution in gender value (see Fig. 2.1), the above position effects can be thought of as representing the strategy of flower production of plants with various sexual phenotypes. However, since the general trend is for plants to be either more male in their gender or more female (see Chapter 2), positional segregation of flowers by sex may also reflect differences in architectural parameters between male and female plants. Plants that tend to be male are taller than those that tend to be female, and male plants produce flowers relatively higher than female plants. As well, either male plants do not have as many higher order branches as female plants, or they do not place their flowers on as high orders as female plants.

Plant architectural effects are most conspicuous in dimorphic sexual phenotypes, such as dioecy where there exist unisexual males and females (Lloyd and Webb 1977). Females in *Rumex acetosa* were taller and had higher canopies than male individuals (Putwain and Harper 1972). Differences in the number of leaves, number of leaves initiated, and shoot production have been reported between males and females of *Rumex acetosella* (Lovett Doust and Lovett Doust 1987). In the dioecious perennial herb *Silene alba*, relative allocation to vegetative and reproductive structures differed between the sexes (Gross and Soule 1981). The growth of both males and females decreased with increasing elevation in *Populus tremuloides*, however, the growth of females decreased at a greater rate

(Grant and Mitton 1979). In absolute terms, females consistently experienced greater radial growth. Female individuals in *Simmondsia chinensis* are morphologically different than males, in leaf size, internode length, and branching frequency (Kohorn 1994).

When the sex habit of a plant is monoecious with male, female and/or hermaphrodite flowers on the same individual, there may be differences in the relative position of flowers of different sexes. In addition to architectural differences between unisexual males and females, *T. hirsuta* individuals that contain more than one flower type may distribute their flowers nonrandomly within a plant. Studies in the relative distribution of flower types in andromonoecious species have revealed differences in location between hermaphrodite and male flowers (Primack and Lloyd 1980, Bertin 1982, O'Brien 1994).

In *Leptospermum scoparium*, there are more hermaphrodite flowers near the top of the plant than towards the base (Primack and Lloyd 1980). In addition, these floral types are more likely to be found in terminal positions on branches of primary and higher orders than proximate positions. *Leptospermum myrsinoides* and *L. continentale* have more hermaphrodite flowers on the outermost sections of branches (further from the main stem) than the innermost (O'Brien 1994). In contrast, Bertin (1982) found hermaphrodite flowers in *Aesculus pavia* located at lower positions and closer to the main stem, more frequently than male flowers. Positional differences have also been reported among male and female flowers in the monoecious cucurbit, *Apodanthera undulata* were observed (Delesalle 1992). Pistillate buds were more commonly found at distal branch nodes, while staminate buds were more frequent at shorter, earlier-produced buds.

Several explanations for differences in flower position have been proposed. These have been based upon the effects of predators and pollinators, dispersal strategies, structural constraints and local resource

availability (Primack and Lloyd 1980, Bertin 1982, Tomlinson 1987, Cox 1988, Waller 1988, Kohorn 1994, O'Brien 1994).

The distribution of male and female strobili in *Taxus canadensis* was found to be dependent upon the effects of deer browsing (Allison 1992). In this species, herbivory is biased towards larger plants that are female-biased, causing the remaining population to be small and male-biased. Bertin (1982) discussed the differential placement of male and hermaphrodite flowers in *Aesculus pavia* in the context of predation as well. He suggested that the vertical distribution of flowers was most likely due to the fact that lower, inner positions suffered the least predation and thereby provided a selection pressure for the placement of hermaphrodites. He also suggested that pistillate flowers should be placed where fruits, especially large fruits, would be structurally stable (Bertin 1982). It is not likely that structural constraints affect the distribution of flowers within *Thymelaea hirsuta*, as the fruits are relatively small and the presence of herbivory has not been studied.

If pollinators are sensitive to the position of flowers on plants, then their behaviour may select for particular within-plant floral patterns (Tomlinson 1987). A conspicuous floral display near the top of a plant may encourage pollen dispersal and consequently increase fitness (Waller 1988). Furthermore, if pollinators visited the base of a plant first, and worked their way up, outcrossing would be favoured when pistillate flowers are near the base of the plant and staminate flowers further up (Bertin 1982). Although neither of these hypotheses have been tested in *T. hirsuta*, they remain viable possibilities.

The most widely cited reason for within-plant spatial patterns of floral types is local resource availability. Primack and Lloyd (1980) suggested that hermaphrodite flowers found higher up on *Leptospermum scoparium* plants than males, enjoyed greater nutrient availability through increased light intensity or apical dominance effects. They also suggested that the 'choice' of each primordium to become a particular sex should be based upon the

reduced cost associated with maleness. In *L. myrsinoides* and *L. continentale*, O'Brien (1994) also determined there was a possibility that "floral buds developing on the outer and upper sections of branches, nearest to the leaves act as immediate sinks to photosynthate and that buds developing further in have reduced access to these resources" (pg. 759).

In *Aesculus pavia*, Bertin (1982) considered the possibility that pistillate flowers were located in particular positions because of local resource availability. Fruit production was largely constrained by light, and thus it would be most favourable for pistillate flowers to be placed at more lighted positions. Bertin (1982) also stated that photosynthate production and acquisition was dependent upon the amount of light energy available, the number of leaves produced, and stem growth.

Although female flowers in *Thymelaea hirsuta* were found closer to the base than males, they were also more likely to appear on higher branch orders. It is possible that these positions are correlated with resource availability through photosynthetic activity in leaves and that distributions of *T. hirsuta* flowers represent local responses to the cost of fruit production. However, it must be noted that further studies in local resource availability within plants are required to determine this conclusively.

### **Environmental Effects on Sex Expression**

This study provides indirect evidence of environmental effects on sex expression in *Thymelaea hirsuta*. Water treatment significantly affected the height of plants, and plant height was highly correlated with a flower's sexual phenotype. Although this evidence may seem contradictory to what was presented earlier, in Chapter 2, where water treatment was not found to significantly affect plant gender, those results were perhaps much less powerful statistically. First, gender values were not distributed normally and so had to be dichotomized when used in the parametric MANOVA procedure (Table 2.5). Secondly, this study included over 12,000 individual flowers,

and so had much higher power than that carried out in Chapter 2.

The plasticity of plant form allows environmental effects to affect reproductive parameters (Tomlinson 1987, Cox 1988, Waller 1988). Waller (1988) cites a simple example, "a monocaulous (single-stemmed) tree may end in an inflorescence, making it necessary to be monocarpic" and consequently "ecological conditions that favour these [monocaulous] architectures could indirectly favour moncarpy" (pg. 209). In the same way, ecological conditions that favour greater height, such as increased water availability, will also favour production of male flowers. However, these results do not support those of El-Keblawy et al. (1995) who noted that populations of *T. hirsuta* that were more male-biased at sites that demonstrated higher aridity in the Mediterranean region of Egypt.

Studies have been carried out that directly link morphology and reproduction in plant species. Lechowicz (1984) examined this relationship in *Xanthium strumarium*. He found that staminate flower and seed production were related to growth in height, as well as to stem, leaf and root biomass allocation. Furthermore, the more branched a plant was, the more staminate inflorescences and seeds were present. Physiological parameters that defined the plants' carbon assimilation and water availability were also important in determining seed output (Lechowicz 1984). Here, environmental conditions affected physiological traits, and thus architecture affected plant reproductive fitness.

In *Apodanthera undulata*, gender was influenced by the same factors that affected growth, suggesting that architecture and sex expression were linked (Delesalle 1992). In *Simmondsia chinensis*, there were significant differences in plant form between males and females (Kohorn 1994). These differences had fitness consequences for each sex, as more extreme morphologies that resembled the opposite sex suffered in terms of favourable seed traits. Kohorn (1994) suggested that morphology was an evolved response to particular resource demands unique to each sex and

that dioecy arose due to "different morphological optima for each sex" (pg. 2384). It is also possible that the evolution of dioecy in *T. hirsuta* is a consequence of selection for divergent morphologies.

## General Discussion

There were two major goals of the present study, examining the reproductive biology of *Thymelaea hirsuta*. The first was to describe the variability in sex expression in this extremely sexually plastic species (see Table 1.1), and the second was to address what factors influence this variability. Although the lability of gender in *T. hirsuta* between reproductive seasons could not be examined as I only took useful data for one year, a detailed examination of within- reproductive season spatial and temporal gender fluctuations was conducted.

Although the results of categorizing the sex expression of this species typologically were complex, it was evident that the majority of plants were either strictly male or strictly female (Table 2.2). Quantitatively, it was more apparent that the *T. hirsuta* population raised in the greenhouse consisted of plants that invested more in either maleness or femaleness, a phenomenon in agreement with Lloyd and Bawa's (1980) notion of sexual dimorphism (Fig. 2.1, 2.2).

I hypothesized that aridity would significantly affect gender in *T. hirsuta*. Although there was no evidence of any direct effects of water treatment on sex expression, there was strong evidence of indirect, architecture-mediated effects caused by water treatment. More specifically, water treatment significantly affected height, and the sex of flowers was significantly influenced by height (see Architecture Study: Discussion). The results of these studies have broad implications for the evolution of dioecy.

### Implications for the Evolution of Dioecy

Typologically, there are several basic gender phenotypes that best describe the spatial and temporal variation in *T. hirsuta* gender. Most individuals are either pure male or pure female, demonstrating very little of

**this variation within a reproductive season (Table 2.2). An additional 16.4% were subdioecious (with 30 subandroecious and 20 subgynoecious individuals out of 305). A smaller proportion were dichogamous, and duodichogamous: 4.3% were protandrous, 3.9% were protogynous, 2.0% demonstrated female duodichogamy and 3.6% male duodichogamy (Table 2.2). It is also important to realize that < 2% of all flowers produced were hermaphrodite, hence most gender phenotypes are the result of spatial and temporal combinations of male and female flowers. However, whether one examines *Thymelaea hirsuta* qualitatively or quantitatively the presence of intermediate forms suggests that this species is in a transitional stage along a continuum that extends to complete dioecy.**

**In the genus *Wikstroemia* the presence of cryptic male and female flowers, morphologically appearing to be hermaphroditic was cited as evidence that hermaphroditism was the ancestral state (Mayer and Charlesworth 1992). In *T. hirsuta*, morphological hermaphrodites may be functionally female-steriles and thus cryptic males (see Chapter 3). In general, most angiosperms are thought to be ancestrally hermaphroditic, and dioecy the derived condition (Lewis 1942, Charlesworth and Charlesworth 1978b). While, it is difficult to know the exact nature of the present selective forces acting on *T. hirsuta* populations, it is assumed that dioecy is the more derived condition. However, at present it is unclear whether dioecy is in fact forming or breaking down in this species (El-Keblawy et al. 1996a).**

**There is probably some degree of inbreeding depression that occurs in *T. hirsuta*, due to the negative effects of geitonogamous pollination ( $\delta = 0.35$ ). The opportunity for male and female flowers on the same plant to 'mate' exists in all plants that show intermediate gender. Temporal changes such as dichogamy or duodichogamy produced both male and female flowers simultaneously at certain times. In addition, male and female phases themselves often show opposite flower function. Although the strength of**

negative inbreeding effects are not in themselves enough to maintain pure female forms ( $\delta > 0.50$ ) the avoidance of outcrossing still contributes to selection for the evolution of dioecy in *T. hirsuta*.

*T. hirsuta* is a woody species with small, inconspicuous flowers that are yellow when male and slightly more green when female. It is a perennial shrub and as it grows older and becomes larger, opportunities for geitonogamy should increase, further selecting for dioecy as a mechanism to reduce inbreeding effects (de Jong et al. 1993). The floral morphology indicates the possibility of pollinator behaviour as another potential mechanism in the evolution of dioecy. *Thymelaea hirsuta* has been reported to be pollinated by entomophily (Dommée et al. 1990, personal observation). An accelerating male gain curve could be produced if generalist pollinators were sensitive to the number of flowers per plant as an attractant and certain plants reallocated female resources to produce male flowers (Bawa 1980, Thomson and Brunet 1990).

There were significant differences in position between flowers of different sexes (Chapter 4). Male flowers were more likely to be produced by taller plants, than female flowers. Male flowers were more likely to be positioned higher on plants, and on branches of lower order than females. If these differences are adaptive then I would suggest that attaining fitness through male and female function requires different optimal conditions, and could be a strong selection pressure for the evolution of different male and female morphs.

### **Implications for Pathways to Dioecy**

There are two main pathways to dioecy hypothesized by previous authors to be relevant to *T. hirsuta*, evolution of dioecy via monoecy and via gynodioecy (Dommée et al. 1995, El-Keblawy et al. 1996a). If sex conversion genes changed the relative number of male and female flowers within a plant then intermediate stages would consist of both male and

female individuals that were variable. However, if a gynodioecious pathway was involved then females would be much less variable than would 'inconstant' males (Lewis 1942, Ross 1970, Charlesworth and Charlesworth 1978a, 1978b, Bawa 1980).

Evidence that *T. hirsuta* intermediates represent individuals that are evolving towards dioecy via a monoecious pathway is derived from quantitative data on gender. The quantitative gender distribution when examined, is symmetrical, suggesting that plants of intermediate gender are variable in their maleness and femaleness to a similar degree. Plants which tend to be female ( $GV > 0.5$ ) are just as likely to have as many male flowers as plants that tend to be male ( $GV < 0.5$ ) have female flowers.

Charlesworth and Charlesworth (1978b) have suggested that this pathway is likely to evolve over a long period of time. Dichogamous forms could be viewed as monoecious individuals selected for a decreased overlap of male and female function, though imperfectly so. Alternatively, the presence of dichogamous forms also supports Dommée et al.'s (1995) heterodichogamous pathway.

The path from hermaphroditism to monoecy requires at least two main steps: a male sterility mutation establishing female flowers, followed by gene mutations that gradually decrease female function of hermaphrodites (Charlesworth and Charlesworth 1978b). Relatively stable female flowers and inconstant male flowers that may appear as hermaphrodites are expected. Only two plants (of 305) that were otherwise exclusively male produced hermaphrodite flowers. No pure female plants produced hermaphrodites, and 18 plants that produced a combination of male and female flowers also produced hermaphrodites. As well, on any particular observation, hermaphrodites were found in combination with males, or males and females but never with only females. This hypothesis is in agreement with the possibility that hermaphrodites are actually cryptic males. Quantitative evidence from greenhouse populations does not support the

idea that female plants are less variable than males. However, field data suggest otherwise, as more hermaphrodites were observed and found to contribute to inconstancy in males. A tradeoff between male and hermaphrodite function also provided evidence for the gynodioecy sequence (El-Keblawy et al. 1996a).

It would be interesting to see how individuals change quantitatively over time, from year to year, in a constant environment. It is possible that there are elements of environmental sex determination occurring in conjunction with genetic effects, making interpretation of observed results difficult. *Thymelaea hirsuta* plants may also be following more than one pathway (El-Keblawy et al. 1996a).

In summary, there are several points that suggest *Thymelaea hirsuta* is intermediate in its evolutionary transition to dioecy: 1) The presence of intermediate gender forms suggest that evolution is still in progress; 2) Results of quantitative gender assessment indicate that most individuals are either more male or more female in their sex expression; 3) The existence of qualitative gender results suggest that the pathway involved may be evolution of dioecy via monoecy; 4) Differences in flower positions among male and female flowers suggest that efficiency of these sexes is achieved through differing morphologies and; 5) The negative effects of geitonogamy suggest that the avoidance of inbreeding depression could also serve as a selection pressure for the evolution of dioecy.

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## **VITA AUCTORIS**

**NAME:** Rita Morbia

**PLACE OF BIRTH:** Rajkot, Gujarat, India

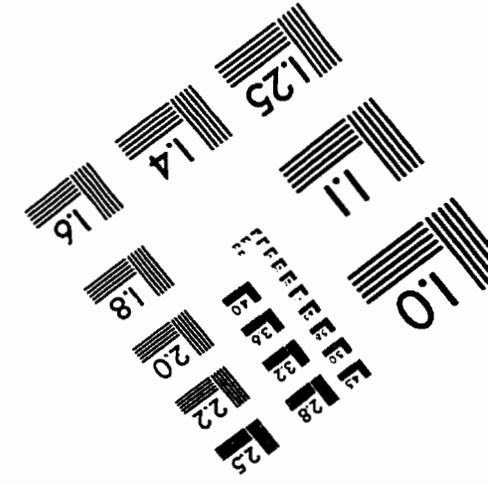
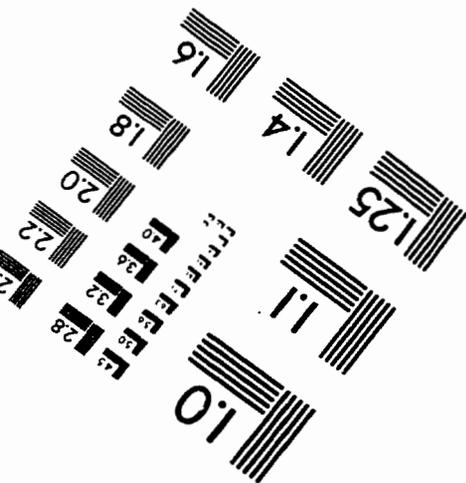
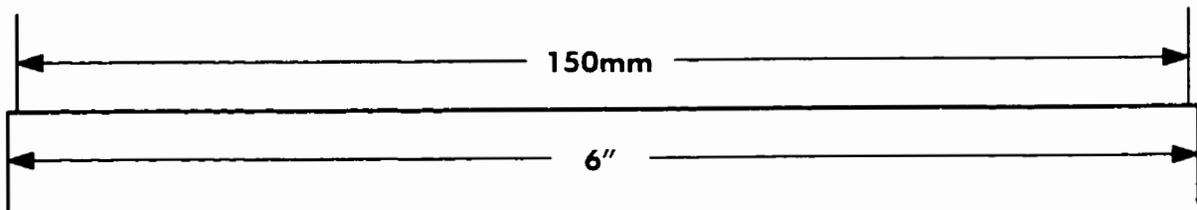
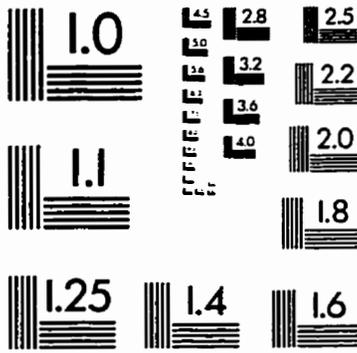
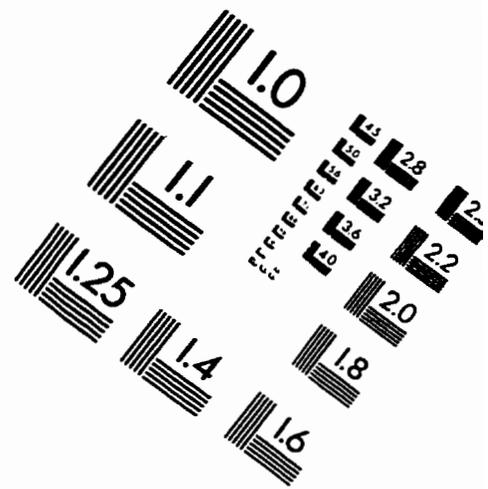
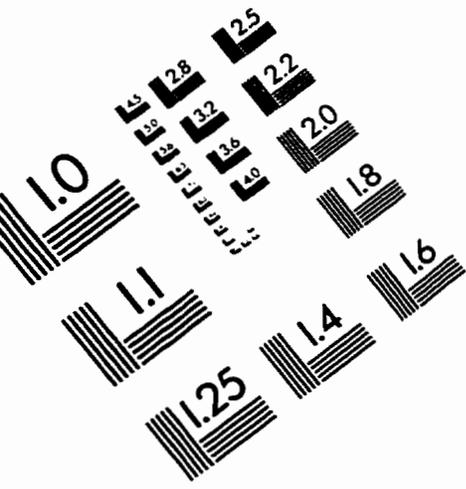
**YEAR OF BIRTH:** 1969

**EDUCATION:** Centennial Secondary School, Windsor, Ontario  
J. L. Forster Secondary School, Windsor, Ontario  
1982 - 1987

McGill University, Montreal, Quebec  
1987 - 1991 B.Sc.

University of Windsor, Windsor, Ontario  
1994 - 1996 M.Sc.

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