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DIOECIOUS PLANTS: EVOLUTION AND SEX RATIO AND ASEPEN DECLINE

by

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DEDICATION

This work and the effort behind this dissertation are dedicated to everyone who helped make it possible.

Thank you.

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CHAPTER 1: BIASED SEX RATIOS IN PLANTS: THEORY AND TRENDS

Introduction

Many dioecious plants display population sex ratios that are biased. In fruit crops the economic importance of sex ratio knowledge is clear. Papaya and dates are dioecious species whose population sex ratio is of direct interest to humans as the production of fruit is the goal. Papaya and date producers should maximize female plants while ensuring that there are enough males to fully pollinate all of the females. In asparagus and spinach, also dioecious crops, the sex ratio is not as important since it is the vegetative part of the plant that humans use. Sex ratios are still important, however, if seed production is required.

Sex ratios are not just important in relation to consumption of plant products by humans; they are important to plant populations genetically. A skewed sex ratio serves to lower the effective population size, which can lead to genetic bottlenecks. This is of special interest, since dioecious plants are thought to have evolved, at least in part, to avoid inbreeding. Sex ratio theory itself is of interest to evolutionary biologists because it provides clear predictions that can be directly tested.

Theory predicts that if costs to produce a male or female offspring are equal, and if males and females differ in reproductive fitness equally with increasing size or age, then natural selection will act to balance a population sex ratio at 1:1 (Charnov, 1982b; Fisher, 1930). Deviations from these assumptions have been explored and the resulting effects on population sex ratio observed, but such analyses have been applied almost exclusively to the animal kingdom. Using studies available in the literature, particularly experimental studies on sex ratios in dioecious plant populations, we examine whether existing theory can account for observed sex ratios and whether this theory, originally developed for animals, needs modification for application to plants.

Sex determination in plants is the focus of many studies (Charlesworth et al., 2005; Liu et al., 2004; Meagher, 2007; Sather et al., 2010). The occurrence of XY chromosomal sex determination and X dosage systems are extremely rare in plants, having been identified in less than 10 species (Ainsworth, 2000). Many species exhibit autosomal sex determination (Grant et al., 1994) and mistletoes have an XO system (Barlow and Wiens, 1976).

In species where sex is not genetically determined, a host of factors can act to influence sex determination and population sex ratio. These include environmental factors (Charnov and Bull, 1977; Freeman et al., 1980; Freeman, 1985; McArthur, 1977; McArthur and Freeman, 1982; Meagher, 1988; Meagher, 1980; Pannell, 1997; Waser, 1984) that impact differential fertilization (Correns, 1928), germination (Eppley, 2001; Freeman, 1985; Purrington, 1993), flowering (or apparent sex ratio) (Allen, 1993; Freeman and McArthur, 1984; Nicotra, 1998; Vernet, 1971), mortality (Eppley, 2001; Freeman and McArthur, 1984; Freeman and Vitale, 1985; Korpelainen, 1993; McLetchie, 1992), or a combination of the above (Lloyd, 1977). The physiological basis for some environmentally based sex determination systems has been worked out (Gregg, 1975). Evolutionary theory is extensive and is believed generally to explain what should happen at the population level under different circumstances (see below), but the specific mechanisms that produce biased sex ratios in nature have received very little attention (but see (Dawson, 1993; Dodson, 1962; Freeman and McArthur, 1984; Gregg, 1975; Meagher, 1981)).

Over 200 published studies on dioecious plants containing sex ratio information were examined for this study. This literature covers 68 families, 175 genera and 250 species. The data used in this analysis was compared with the data in Renner and Ricklefts' 1995 overview of dioecious plant families of the world (Table 1.1). With respect to geographic location, life form, and pollinator (the three categories considered by Renner and Ricklefts, 1995), the families studied do not form a random sample of the whole. For instance, trees are overrepresented in the literature, as are temperate region families, while underrepresented categories include animal pollinated families and shrubs. Some families are overrepresented as well in the number of species that have been studied. Examples of highly studied families are Salicaceae and Cucurbitaceae, while many families only have one species represented. This skewed sampling may influence the conclusions derived from our analysis.

Theory

Sex ratio theory is deeply rooted in zoology (Charnov, 1982b; Fisher, 1930; Ghiselin, 1969; Hamilton, 1967). Fisher (1930) provided the foundation for this theory, explicitly predicting that:

1. Given equal reproductive costs to produce a female versus a male offspring, natural selection will act to balance the sex ratio of the population at unity.
2. If one of the sexes is cheaper to produce than the other, then parents with a tendency to overproduce the cheaper sex will acquire a larger inclusive fitness.
3. The rarer sex at time of reproduction is the more valuable. As a result, a female overproducing that sex will have greater genetic representation in the next generation (larger reproductive fitness) than a female who does not overproduce the rarer sex.

Fisher’s predictions rely on the fact that in sexual reproduction every individual receives half of its nuclear genetic information from its mother and half from its father (i.e., equal input from each sex). The predictions also assume that resources are limited, that there is some upper bound to the number of offspring an individual can produce, and that the population is panmictic (Fisher, 1930).

According to Shaw and Mohler (1953), the contribution of a single progeny male (C_m), (the same can be calculated for a female (C_f)), to the grandchildren's generation (G_2) is:

$$C_m = C_f \equiv C = \left(\frac{n}{4N}\right)\left[\frac{x}{X} + \frac{1-x}{1-X}\right] \qquad (1)$$

where n = number of zygotes per individual, $N = \Sigma n$ = total number of zygotes in a population, x = proportion of males (females) produced by an individual, X = total proportion of males (females) produced by the population (Shaw and Mohler, 1953).

N and n can be assumed to both be positive and constant, so that C is a function of x and X. Further, the factor $n/4N$ can be ignored since it will not change the nature of the conclusions. It is also important to note that both x and $X \in [0,1]$. Thus, equation (1) can be modified:

$$\begin{aligned} C(x,X) &= \frac{x}{X} + \frac{1-x}{1-X} \\ &= x\left(\frac{1}{X} - \frac{1}{1-X}\right) + \frac{1}{1-X} \qquad (2) \end{aligned}$$

To determine the strategy which maximizes the contribution (C) of an individual, three cases must be addressed: when males are in equal proportion to females (X = ½), when males outnumber females (X > ½), and when females outnumber males (X < ½).

Case 1: X = ½

Substitution of X = ½ into equation (2) results in C = 2. This is important because as Shaw and Mohler (1953) pointed out, it does not depend on x (the sex ratio of the individual's offspring). As illustrated in Figure 1, as long as the population as a whole has a 1:1 sex ratio (X

= ½), an individual can produce any progeny sex ratio and be equally as fit as if they produced any other ratio. Thus, there is no penalty or reward for producing a male- (or female-) biased progeny sex ratio as long as another individual produces an equally biased female (or male) progeny sex ratio, keeping the population sex ratio at unity.

Case 2: $X > ½$

If $X > ½$, then $\frac{1}{X} < \frac{1}{1-X}$, and equation (2) becomes:

$$C = x * (\text{negative \#}) + \text{constant.}$$

Therefore, C is maximized when $x = 0$. Thus, if the population has a male-biased ratio, then the individual can maximize its contribution by producing all females.

Case 3: $X < ½$

If $X < ½$, then $\frac{1}{X} > \frac{1}{1-X}$, and equation (2) becomes:

$$C = x * (\text{positive \#}) + \text{constant}$$

Thus C is maximized when $x = 1$. In other words, if the population has a female-biased sex ratio then the individual can maximize its contribution by producing all males.

This supports Fisher's theory on the benefits of overproducing the rarer sex, which was also supported by Shaw and Mohler (1953). Plotting equation (1) against X produces a series of curves for different values of x, visually confirming the results above (Figure 1.1).

Survivorship

There are two types of survivorship that are of interest. There is seed or seedling survivorship through the end of parental investment and there is plant survivorship after the period of parental investment.

If the survivorship of males and females through the end of parental investment are different, but consistent through the population, then the terms simply cancel out ($s_m/S_m = 1$), and there is no effect on the genetic contribution of the parent in question (Shaw and Mohler, 1953).

As Shaw and Mohler (1953) show, incorporating survivorship (s_m and s_f for the survivorship of males and females through the end of parental investment produced by the individual of interest, and S_m and S_f for the survivorship of males and females through the end of parental investment produced by the population as a whole) into equation (1) produces:

$$C = \left(\frac{nxs_m}{2NXS_m}\right) + \left(\frac{n(1-x)s_f}{2N(1-X)S_f}\right) \qquad (3)$$

If the survivorship values are constant for the population, then $s_f = S_f$ and $s_m = S_m$, and:

$$C = \left(\frac{n}{2N}\right)\left[\frac{x}{X} + \frac{1-x}{1-X}\right]$$

This equation differs from the equation derived when survivorship is not considered only by the constant ½, which is only the difference between contribution to the F1 generation and the F2 generation which was the original consideration. If however, $s_f \neq S_f$ and $s_m \neq S_m$ then, equation (3) is a more accurate definition of contribution than equation (1). This effect can alter results in a number of ways, all of which depend on the relative survivorship values in addition to the values of x and X. One example is to consider a population where the primary sex ratio is initially 1:2 ($X = 1/3$), and in general, equal numbers of males and females survive ($S_m = S_f \equiv S$). Then equation (3) becomes:

$$C = \left(\frac{3n}{4NS}\right)\left[2xs_m + (1-x)s_f\right] \qquad (4)$$

If $x= ½$ (for convenience) then:

$$C = \left(\frac{3n}{4NS}\right)\left[s_m + \frac{1}{2}s_f\right] \qquad (5)$$

It is clear that C is maximal when both s_m and s_f equal 1, (i.e., all offspring survive). But it is also clear that the survivorship of the females has half as much weight as the survivorship of the males. This is because males are underrepresented in the population ($X < ½$). In the same fashion, if $X = 2/3$, (a male-bias ratio), then:

$$C = \left(\frac{3n}{4NS}\right)\left[\frac{1}{2}s_m + s_f\right] \qquad (6)$$

In this case the female survivorship is more heavily weighted.

Annual plants have a single production of seeds, the survival of which is easy to calculate. Perennial plants produce offspring several different times throughout their life, complicating the calculations for both number and survival of offspring produced. In this case, the number of offspring and their survivorship must be summed over the life of the plant, keeping in mind that seed production and quality might vary in response to factors such as the environment, age of plant, and previous production.

Survivorship is a highly important factor in determining sex ratios. Evolutionarily, it is the sex ratio at the time of reproduction that influences Fisher's theory, not the primary sex ratio. This implies that differential survivorship, differential pollination, or differential flowering by males and females can profoundly influence the contribution that individuals make to the next generation (Meagher, 1981; Taylor et al., 1999b).

Taylor et al. (1999a) report on an example of the influence a skewed sex ratio can have on the progeny sex ratio in *Silene*. A single male pollinator used to pollinate flowers on a female plant produced a significantly female-biased progeny ratio (34% male). A small pollen load signifies that there are few males reproducing; whether it is due to timing, production, or number. An increase in male flower production typically increases the pollen available. This study showed that there was a direct influence on severity of sex ratio by availability of pollen (Taylor et al., 1999a), illustrating how crucial the secondary sex ratio, and potentially survivorship of one sex compared to the other, can be to future sex ratios of a species.

The second form of survivorship comes after parental investment and involves the differential allocation of resources to reproduction (Delph, 1990; Gross and Soule, 1981; Korpelainen, 1992; Queenborough et al., 2007; Wallace and Rundel, 1979) (Table 2). Reproducing as a male is limited to pollen production (and perianth production in animal pollinated plants), while females must produce seeds and fruits (and the perianth in animal pollinated species). In the herbaceous plant *Silene alba,* females allocate more resources to reproduction than males over the course of a season as long as they have at least a 20% fruit set (Gross and Soule, 1981). As is often the case, males allocate more resources to flowering than females, since although male flowers are often smaller than female flowers, males produce significantly more flowers (Armstrong and Irvine, 1989; Delph, 1990; Gross and Soule, 1981). By allocating fewer resources to flowering, females can allocate these early resources to growth and maintenance, which could be beneficial for setting seed later in the season (Gross and Soule, 1981). These types of differential cost are usually measured using biomass of the reproductive structures or products, but other measures such as amounts of limiting nutrients can be used as well. In one study different concentrations of chlorophyll were measured in the leaves of male and female individuals of fourwing saltbush (Tiedemann et al., 1987). Thus, males and females may also differ in their ability to carry out photosynthesis.

Not only is there a difference in the biomass/nutrients/energy associated with reproducing as one sex or the other (Table 1.2), but there is a temporal difference as well. The most demanding portion of male reproduction takes place earlier than seed and fruit production, which are invariably the most resource intensive aspects of female reproduction. For example, in their study of plant water stress, Freeman and McArthur (1982) found no difference in the water stress of male and female *Atriplex canescens* (a desert shrub) in June when they flower, but that females became progressively more stressed as they matured their fruits in July and August.

Although the theory is established (Charnov, 1982b; Levins, 1968), it is difficult to quantify a differential resource term (*R*). Since the differentiation is incurred at the plant level, *R* is assigned to the contribution as a whole, not broken up in terms of offspring. Thus:

$$Cm = Rm\left(\frac{n}{2N}\right)\left[\frac{xm}{XM} + \frac{(1-x)f}{(1-X)F}\right] \quad \text{and} \quad Cf = Rf\left(\frac{n}{2N}\right)\left[\frac{xm}{XM} + \frac{(1-x)f}{(1-X)F}\right] \qquad (7)$$

where *m*, *M*, *f*, and *F* are the male and female progeny and population terms respectively, composed of many variables, survivorship and cost being the two discussed above.

R is left undefined in the literature, but possible definitions include: biomass (Table 1.2 references), limiting nutrient concentrations (Antos and Allen, 1990; Wallace and Rundel, 1979), water stress (Freeman and McArthur, 1982), energy (Antos and Allen, 1990; Smith and Evenson, 1978), or a relative term such as tradeoff between reproduction and future growth and reproduction (Ashman, 1994; Charlesworth and Charlesworth, 1987a; Horvitz and Schemske, 1988; Reznick, 1985). Obeso (2002) shows that researchers agree that some trade off of resources must occur but it is exceedingly difficult to predict how this trade off may work. Difficulties in detecting the costs of reproduction include: differential input of resources, weak trade-offs, and resource storage abilities (Obeso 2002). Additionally, the relationship between genetic contribution and cost of reproduction is most likely non-linear. Therefore, *R* should be regarded as an unknown function rather than a percent.

Cost

One of the original assumptions of Fisher (1930) was that male and female offspring cost the same to produce. Hamilton (1967) proposed that this need not be the case, and that differential costs would lead to an over-representation of the cheaper sex, assuming that equal parental efforts were given to each of the sexes.

Quantitatively, differential offspring costs can be considered in the same way as differential survivorship. If the cost of a male is constant throughout the population, cost of progeny male (e_m) = cost of a general male in the population (E_m), and similarly for females, $e_f = E_f$, then as with survivorship, these values cancel out and equation (1) is left unchanged. If these values are different, it implies that the cost incurred by the target plant is different from the cost incurred by the general population. This could be due to differential resource availability or utilization. If this is the case, one of the values is set to one and the others are normalized around that value. If the reproductive cost of a male in the population is set to 1 ($E_m = 1$), then E_f, e_m, and e_f are given cost values as they relate to E_m. The equation is given by:

$$C = \left(\frac{n}{2N}\right)\left[\frac{x\left(\frac{1}{e_m}\right)}{X} + \frac{(1-x)\left(\frac{1}{e_f}\right)}{(1-X)\left(\frac{1}{E_f}\right)}\right] \qquad (8)$$

This looks similar to the result achieved when considering survivorship, however, n, and as a consequence N, are no longer constant. Assuming that resources are held constant, the number of offspring will increase as the less expensive sex is overproduced. Combining differential survivorship and differential cost of the sexes we arrive at:

$$C = \left(\frac{n}{2N}\right)\left[\frac{x(s_m)}{X(S_m)(e_m)} + \frac{(1-x)(s_f)(E_f)}{(1-X)(S_f)(e_f)}\right] \qquad (9)$$

To illustrate the effects substitute $X = 1/3$, $S_m = S_f = S$, $E_m = E_f$, and $x = ½$ to get:

$$C = \left(\frac{3n}{4NS}\right)\left[\frac{s_m}{e_m} + \frac{1}{2}\frac{s_f}{e_f}\right] \qquad (10)$$

As was seen in survivorship, the term in square brackets associated with the underrepresented sex carries more weight than that of the overrepresented sex, but this time the term is a function of survivorship and cost. Intuitively, this pattern (a variable that affects male and female progeny differently) can now extend to as many variables as necessary. Thus, terms can be added to the numerator and denominator corresponding to the variable(s) of concern.

Within the seed plants, differential cost would imply that more of a limiting resource was required to produce a seed destined to yield one sex than the other sex. In more primitive heterosporous plants, differential cost would mean that the allocations of limiting resources to megaspores and microspores were not the same. Little empirical data has been collected in this area. Most seed dimorphisms occur among annual plants (Berger, 1985; Gardocki, 2000; Redondo-Gomez et al., 2008; Telenius and Torstensson, 1988; Wang et al., 2008) and there are relatively few annual dioecious plants (Bawa, 1980; Renner and Ricklefs, 1995). In perennial dioecious plants there is almost always a long delay between seed germination and production of sex organs. Thus, the difference in the cost in parental allocation probably pales in comparison to differences in microhabitat. Nevertheless, there are annual dioecious plants that can be used to explore the issue of differential cost.

Freeman et al. (1994) observed that, in spinach, large seeds (a standard deviation or more above the mean weight) disproportionately resulted in male offspring. In the wet environment the *m/f* sex ratio was 1.5 while in the dry environment it was greater than 2.5. Conversely, the smaller seeds (a standard deviation or more below the mean weight) disproportionately resulted in female offspring. Here, the wet environment had a *m/f* sex ratio of 0.75 while in the dry environment the ratio was slightly greater than unity. Making a seed large did not make it male, but male spinach seeds tended to be a more active sink for resources (pulled more resources from the mother plant), resulting in larger seeds and thus a higher cost for male production. As seed size increased, so too did stamen production of the resulting male plant. However, pistil and fruit production were independent of the size of seed the female plant came from. Thus, this is also an example of a population that might display healthy mothers producing more males, as there is a carryover from seed size to adult reproductive potential (Trivers and Willard 1973).

Inbreeding

An inbred population by definition has many genes shared among the individuals within that population. Since the benefit of mating is to pass on genes, it is counterproductive for an inbred population to compete for mates. As a result, as long as there is a sufficient number of males to pollinate all the females, an individual can increase its *C* value by overproducing females (Hamilton, 1967). The extent to which this occurs should be related to the inbreeding coefficient (Charlesworth and Charlesworth, 1987b; Husband, 1996).

Due to plants' inability to move, when pollen and seed dispersal are limited, the population should be inbred and a female bias sex ratio will likely evolve (De Jong et al., 2002). In DeJong et al.'s model (2002), when seed dispersal and pollen dispersal are minimized (the dispersal of both is limited to one unit from the source), there is a slight female bias (*S.R.*= 0.467). As the dispersal distance is increased for both seed and pollen or just for pollen, the population sex ratio increases, becoming more equal or male biased (Bailey and McCauley, 2005). This likely drives major trends in plant sex ratios, as discussed below.

Sex Change

Studies suggest that under life histories providing an increase in fertility with size and/or age, sex change should occur (Charnov, 1982a; Ghiselin, 1969; Leigh et al., 1976). This change in sexual orientation over time is known as sequential hermaphroditism. A change in sex comes at a cost to the individual. New sex organs must be developed or activated and this initial cost must be compensated for by the gain in fitness accompanied by becoming the opposite sex (Leigh et al., 1976). In plants this phenomenon is not uncommon (Bertin, 1993; Freeman et al., 1980; Sprengel, 1793; Stout, 1928). A striking example is *Arisaema triphyllum,* where females have a higher gain in reproductive success with increasing size than males do. Natural populations of *A. triphyllum* reflect this with a higher proportion of females in the upper size class and a higher proportion of males in the lower size class (Policansky, 1981). A unique feature of this perennial herb is its ability to change its sex and size on a seasonal basis (Policansky, 1981). Policansky studied over 1000 individuals (597 male, 312 female and 343 asexual) with respect to reproductive success over three consecutive years. He concluded that to maximize reproductive success *A. triphyllum* individuals should be male at a height less than 398mm and female at a height greater than 398 mm.

Another example of a mechanism leading to a sequential hermaphrodite through changes in reproductive success can be seen in some wind-pollinated species. A male plant benefits from accessing wind currents that will disperse pollen, while female plants benefit from still air that allows pollen to land and adhere to its stigma – tasks which are optimized under very different physical forms (Nicklas, 1985). The male plant is ideally tall and slender, with a high surface to volume ratio (fractal). The female plant on the other hand is optimized at a low surface to volume ratio; accomplished by being short and thick. If as a plant grows it changes from one of these forms to the other, then sexual lability would optimize fitness (Bickel, 1993). In fact, sexual lability is common in many wind-pollinated plants (Charnov and Bull, 1977; Freeman et al., 1980; Korpelainen, 1998). An important note is that since sexual lability can affect the apparent sex ratio, it is important to consider the length of study period in these known species when attempting to determine the sex ratio of the population.

Causal agents of sex change include age (size), temperature, water availability, mites, light, nitrogen, potassium, carbon monoxide, photoperiod, and trauma. In most cases, environmental stresses induced maleness. In cases where female reproduction costs are higher than that of a male, this change could increase individual survival through harsh environmental conditions (Freeman et al., 1980). In some species, like *A. canescens*, not all individuals are capable of changing sex (unlike *A. triphyllum*). In this species only 20% of the individuals are capable of making the sexual switch as a response to stress (Freeman et al., 1984), a capability that is genetically controlled (McArthur et al., 1992).

Environmental Factors

Plants, like animals, are subject to outside factors that can influence both their primary and secondary sex ratios. Examples include availability of resources such as water, nutrients, light, elevation, and salinity. In annual dioecious plants, aspects of environmental quality can differentially influence the gender expressed at flowering (Freeman and Vitale, 1985). Spinach plants raised under water restriction displayed a male-biased sex ratio while plants raised under sufficient water conditions displayed a more even or female-biased sex ratio (Table 3). Similar results have been seen in spinach across a salinity gradient (Vitale et al., 1987).

Environmental effects on sex ratio can result in spatial segregation of the sexes along the environmental gradients causing them. Spatial separation of the sexes has been widely observed (Cole, 1979; Davey, 1917; Dawson, 1993; Dawson and Bliss, 1989; Dodson, 1962; Eppley, 2001; Freeman et al., 1976; Grant, 1979; Lovett Doust and Cavers, 1982; Lysova, 1975; Richards, 1975; Waser, 1984). Mechanisms for spatial segregation have been speculated, and include intraspecific competition avoidance (Freeman et al., 1976), differential mortality (Lloyd, 1977; Meagher, 1981), sex choice (Charnov, 1982a), and maternal adjustment of progeny sex ratio (Charnov, 1982b). Other mechanisms are possible but have little or no evidence to support them (Bierzychudek and Eckhart, 1988). Spatial segregation of the sexes can cause apparent but false discrepancies in observed sex ratios if one microsite is exclusively or disproportionately considered. For example, in Eppley's (2001) study of *Distichlis spicata*, populations of this wind pollinated grass were studied over a nutrient and water gradient. Spatial segregation of the sexes was observed with the majority male (male-biased) sites at significantly higher elevations than the majority female (female-biased) sites. In the Eppley study, elevation is correlated with the factors of interest since lower elevation is indicative of both higher nutrient and water availability. Had elevation not been considered, or the geographic range of sites been smaller, not only would the phenomenon of spatial segregation not have been observed but the estimated sex ratio would have been incorrect.

The spatial segregation of the sexes should influence the rates of inbreeding, but we are unaware of any study that has examined this. One would expect that this could be most important for species where pollen and seed dispersal are limited (e.g., wind-pollinated shrubs with abiotically dispersed seeds).

Environmental effects can even occur across generations. For example in animal populations where males are required to compete for mates and there is a carryover from birth weight to adult size, females in good condition should produce proportionately more males, while females in poor condition should produce females (Trivers and Willard, 1973). In such populations a female is likely to reproduce no matter what her physical size. A small male, however, will likely never reproduce (although in some species "sneaky" males are still successful reproducers), while a large male can readily compete for mates and could reproduce many times, greatly increasing his fitness and his mother's inclusive fitness. Examples are seen in nature, most commonly in animal populations that exhibit harems (Arnbom et al., 1994). There is, however, at least one report of the Trivers-Willard effect in plants. In spinach, larger seeds are disproportionately male, while small seeds are disproportionately female (Freeman et al., 1994). The study also showed a positive correlation between seed size and adult size in males. Because spinach grows in a columnar fashion, a bigger plant is synonymous with a taller plant. As a result, large seeds lead to taller plants and because spinach is wind pollinated, the increase in height has the ability to increase fitness through non-linear expansion of the pollen shadow. Therefore a mother in good condition, capable of producing large seeds, should in theory produce male-biased progeny as their fitness will disproportionately increase with seed size.

Methods

Two hundred and fifty studies of dioecious plants containing sex ratio information were reviewed from the literature. The species were categorized according to life form (herb, vine, shrub, or tree), pollinating agent (insect, animal, or wind), fruit/seed dispersal agent (biotic or abiotic), and sex ratio (male-biased, female-biased, or no bias) (See Table 1.4 for a subset of this spreadsheet). A general log-linear analysis (using software SPSS v18) was then used to determine any significant effects of life form, pollinating agent, dispersal agent, and sex ratio.

Loglinear analysis is used to analyze the relationship between three or more discrete, categorical variables based on cell frequencies. Loglinear models treat all variables as response variables and so no dependent/independent relationships are considered. As a result, models look at the main and interaction effects between variables only. Significance is determined by comparing reduced models to the saturated model (the model obtained when every effect, main and interacting, is considered) (Kennedy, 1992).

For this study, analysis utilized the four main variables stated above, broken into a total of 12 categories. When considered in combination, the contingency table therefore has 72 possible combinations, or cells (4 life forms*3 pollinating agents*2 dispersal agents*3 sex ratio outcomes). The 250 species were then classified into one of the 72 cells based on their specific characteristics (Table 5).

To aid in evaluating our results with theory, peak (modal) and maximum distances for each type of dispersal agent were recorded when available in the literature (Table 1.6). The conversion from agent to distance is required as distance is the measure used by theoretical models (De Jong et al., 2002). Particularly important is the ability to relate pollen versus seed dispersal distances and thus to infer the degree of inbreeding in a population.

Results

The most parsimonious model not significantly different from the saturated model involved only two variables: life form*pollinator*sex ratio and life form*disperser*sex ratio. These variables represent the interaction effect between life form, pollinator and sex ratio, and the interaction effect between life form, fruit disperser and sex ratio. The model fits the saturated model with a p-value of 0.868 indicating that these two variables account for the majority of the weight of the saturated model. As predicted by De Jong et al., both seed and pollen dispersal are involved.

From the frequencies of the contingency table cells (Table 1.5) some trends can be seen (Figure 1.2):

1. Vines are almost exclusively male-biased. 18 out of 19 vine species are insect pollinated, and 17 of the 18 (89%) display a male-bias.
2. Eighty-one percent of insect-pollinated, biotically dispersed tree species display a male biased sex ratio. Conversely, for abiotically dispersed, insect pollinated trees there are almost as many species displaying female-biased ratios as there are species that display male bias.
3. Three of the four shrub categories have more male biased species than female-biased species.
    a. Insect pollinated, biotic fruit dispersal: 51% of the species are male-biased.
    b. Insect pollinated, abiotic fruit dispersal: 69% of the species are male-biased.
    c. Wind-pollinated, biotic fruit dispersal: 64% of the species are male-biased.
    d. Wind-pollinated, abiotic fruit dispersal: 39% of the species are male-biased.
4. Herbs show a general tendency for female-biased sex ratios; the exception being insect-pollinated, abiotically dispersed fruits, of which 58% of the species show a male-bias.
5. Wind-pollinated herbs all tend to be female-bias; especially notable is abiotic fruit dispersal with 61% of the species having female-biased populations.

When considered as a whole, these data suggest that predictions about expected population sex ratios can be based, with some accuracy, on only pollen and seed dispersal distances.

To ensure that genetics is not the underlying factor, the data was categorized according to family and reevaluated. 56% of the families studied were represented by multiple species. 33% of these families were monotypic, meaning each species within shared the same characteristics in all three categories. In this group characteristics and heredity are completely confounding. However, 64% of families with multiple species display different sex ratios including 32% that have both male and female biased species. Some of this is due to sex change or environmental sex determination. This argues that sex ratio is not necessarily genetically fixed within a family. But more importantly, of the non-monotypic families, 87% display multiple sex ratio biases including 39% displaying both male and female biases.

Interpretation

Because plants are rooted in space, it is expected that inbreeding plays a major role in determining population sex ratios. Accordingly, we hypothesized that species with low pollen and seed dispersal distances will likely be inbred and therefore display a female-biased ratio. As the pollen and seed dispersal distances increase, it is expected that population sex ratios will tend towards neutrality. As pollen, but especially seeds, are more widely dispersed, the population density should decline, decreasing the number of neighbors available for mating. This increases the risk of being out of pollination range – a reproductive disadvantage. By this reasoning males will be scarce and a male-biased ratio would therefore increase the chance of a female being located within the pollen shadow in a low-density neighborhood.

Vines:

Vines typically display male-bias sex ratios. Almost all the vines studied are **insect-pollinated and biotically-dispersed**. Most insect pollination is relatively local (< 100m), while bird and mammal dispersed seeds are among the most widely dispersed (> 400m). Therefore, there is reason to believe that dioecious vine populations are male biased to increase the likelihood of successful pollination as males should appear to be scarce.

Trees:

Trees display tendencies toward male-biased populations. The majority of the tree species are **insect-pollinated, biotically-dispersed** and so the argument is parallel to that of the vines; Insect pollination is often more local than biotic seed dispersal, especially bird or animal dispersal.

Herbs:

Insect-pollinated, abiotically-dispersed herbs have a tendency to be male-biased. This contradicts the theory, assuming that insect-pollination and abiotic seed dispersal are both low. Perhaps this can be reconciled by noting that insect-pollination distance could be higher than abiotic seed dispersal given the small stature of most herbs. More specifically, the type of insect-pollinator must be considered more closely (to be discussed later).

Insect-pollinated, biotically-dispersed herbs and **wind-pollinated, biotically-dispersed** herbs have more populations displaying female-bias population sex ratios. Both cases contradict the theory considering pollination is occurring at a fairly local level (i.e., windborne pollen falls off exponentially with distance and wind-pollinated herbs tend to be short), and biotic seed dispersers tend to have larger dispersal distances (Table 1.6).

Shrubs:

In the study sample, shrubs are the most diverse life form with respect to pollinator and dispersal agents, and although they too display a tendency towards male-bias, it is not as obvious.

Insect-pollinated, biotically-dispersed shrubs and **wind-pollinated biotically-dispersed** shrubs show more male-bias populations. This follows the hypothesis, assuming that biotic seed dispersal distances are greater than either wind or insect pollen dispersal distances and hence males appear to be the rarer sex—at least from the female's perspective.

Insect-pollinated, abiotically-dispersed shrubs tend to have male-bias population sex ratios. These conditions imply low pollen and seed dispersal distances, which are indicative of female-bias populations according to the theory. It is possible however, that the implied distances are not correct (discussed in more detail below).

Wind-pollinated, abiotically-dispersed shrub populations were more likely to display a female-bias sex ratio. This is in accordance with the theory of low pollen dispersal and low seed dispersal resulting in an inbred population.

Discussion

Log-linear analysis assumes that the user has good reason to believe that the categories included in the analysis are necessary and sufficient to explain the cell frequencies. We have made a great effort to examine the theory and consider which categories are important. We have included life form, pollinating agent, dispersal agent and sex ratio. The detail with which these categories can be examined depends on available data. For instance, we separated dispersal agent into biotic and abiotic subcategories because the lack of available data restricted further categorical breakdown. The importance of categorical restriction lies in differences encountered within each group. While windborne pollen exhibits an exponential decay away from the source, insect pollen dispersal may vary greatly depending on insect type. Beetles, flies, and many other insects tend to pollinate locally. Honeybees, most moths and butterflies have a somewhat larger pollination range while African bees and sphinx moths are known to spread pollen over many kilometers (Dick, 2003; Janzen, 1971). Clearly the analysis would benefit from further subdivision of insect pollination into at least three types based on pollen dispersal distances, and hopefully, in time, enough data will be collected to allow for such a breakdown. Similarly, abiotic dispersal can vary based on specialized fruit form. Some fruits will simply fall to the ground beneath the parent plant, while others have wings or parachute-like appendages allowing the fruit to cover a much greater distance. As it stands, we are analyzing all types of insect pollination together, as we do for abiotic fruit dispersal and again for biotic fruit dispersal. The fact that we are still able to obtain meaningful results is a sign that trends in biased sex ratios can be both understood and predicted in light of the theory and parameters we have considered.

An additional category, geographic location, specifically tropical versus temperate species, could be examined. Convergent evolution could be responsible for similar sex ratio biases in similar conditions, but a lack of data forced the omission of this category entirely. Ideally, when performing a loglinear analysis an adequate sample size is at least five times the number of cells in the contingency table. For this study, a desired *n* is 360 data points. An *n* of 250 results in a reduced power of the test. This can be compensated for by increasing sample size, or by eliminating categories. An analysis was done after removing animal pollinator, reducing the total number of cells to 48. The elimination of this category also reduced the number of data points to 246, which meets the minimum number of data points required by a 48 cell table. In this case, the most parsimonious model which significantly fit the saturated model consisted of three, three way interaction terms: lifeform*pollinator*sex ratio + lifeform*disperser*sex ratio + pollinator*disperser*sex ratio, and resulted in a fit with a p-value of 0.455. This suggests that while eliminating categories improved the overall power of our test, it also caused a loss in model resolution.

Conclusion

Upon assigning species to one of 72 categories based on four life history traits, we were able to examine combinations that resulted in male, female or no population sex ratio bias. In most cases specific categories were in accordance with the existing theory (most insect pollinated vines with bird or mammal dispersed fruits have male biased sex ratios), although exceptions exist (many herbs which are insect pollinated and have abiotically dispersed fruits have male biased sex ratios—when we would expect female-biased sex ratios). Explanations for these exceptions can only be hypothesized at this point due to a general lack of data. Clearly, what is needed is detailed information on pollen and seed dispersal distances and the degree of inbreeding. Based on a general loglinear analysis of the data set, two three-way interaction effects were found to be most important in estimating cell frequencies. These effects were the interaction between life form, pollinator, and sex ratio, and the interaction between life form, disperser and sex ratio. The existing theory allows us to make specific predictions, such as, insect pollinated, abiotically dispersed shrubs with male biased sex ratios should have much lower rates of inbreeding and higher fruit dispersal distances than plants with similar female biased sex ratios.

CHAPTER 2: INCEST AND INTERACTIONS: THE IMPORTANCE OF MULTIPLE VARIABLE INTERACTIONS IN THE EVOLUTION OF DIOECY

Introduction

Why hermaphroditic plants should evolve a separate sexed mating system (dioecy) has received considerable study beginning with Darwin (1877) and continuing to present (Charlesworth and Charlesworth 1978, Webb 1979, Givnish 1980, Cox 1981, Freeman et al. 1997, Ashman 1999, Heilbuth et al 2001, Renner and Won 2001, Barrett 2002, Charlesworth 2006, Pannell and Verdu 2006). Among the most widely accepted drivers of dioecy are the negative fitness effects of inbreeding (Lloyd 1973, Charlesworth and Charlesworth 1978), physical specialization of unisexual individuals (Darwin 1877, Walace and Rundel 1979, Freeman et al. 1997), including dispersal abilities (Givnish 1980, Dejong et al. 2002), and an increased production of one gamete in response to the loss of the other (compensation) (Eckhart 1992, Poot 1997, Ashman 1999).

Researchers are divided into two schools of thought regarding the evolution of dioecy: a means to avoid inbreeding or to allow for specialization of the two sexual functions. Evidence that inbreeding depression occurs and is selected against is strong (Schemske and Lande 1985, Ashman 2006). However, many outcrossing mechanisms less extreme than dioecy exist (e.g. self-incompatibility, dichogamy, where a flower will mature the different sexual functions at different times, and herkogamy, where the male and female sexual structures are spatially separated), and occur in species that are ancestral to dioecious species (Ornduff, 1966, Freeman et al. 1997, Guibert et al. 2009) thus calling into question the primacy of outcrossing as the driving force behind the evolution of dioecy. Similarly, specialization in the form of flower location within the plant exists in some monoecious species such as *Sagittaria trifolia* (Huang et al. 2002) where flowers at the base of the raceme are female and flowers at the top of the raceme are male. Sexually dimorphic flower size is another type of specialization which occurs in monoecious and dioecious species. For instance, many temperate, animal-pollinated species have larger male flowers compared to female flowers (Delph et al. 1996, Yakimowski et al. 2011).

Current models of the evolution of dioecy examine the role of a particular pressure on the evolutionary process. Examples include: inbreeding depression (Lloyd 1973, Charlesworth and Charlesworth 1978), outcrossing (Bawa 1980, Thompson and Barrett 1981), geitenogamy (Dejong et al. 1993), and pollen and seed dispersal ability (Givnish 1980, Heilbuth et al. 2001, Dejong et al. 2002). Most of these models examine the effects of only one or two factors. In nature many of these factors (and likely more) are operating at once. Here we examine the role of compensation, inbreeding depression, and specialization independently and in combination, on a unisexual mutant’s ability to invade a hermaphrodite population. The goal is to identify the conditions that make unisexual invasion possible.

Methods

Model Development and Assumptions

We created a model with an extensive user interface (Image 1), allowing the user to select and manipulate multiple variables. One such variable allows the user to select between a model based on genetically determined offspring, and one based on phenotypically determined offspring. Both models assume that: 1) the population is partially self-fertilizing; 2) two mutations are required to evolve dioecy from a hermaphroditic state (one that causes male sterility and one that causes female sterility). The models differ only in how the sex of the offspring is determined. The genetically determined model assumes both mutations are dominant and that loci for pollen and seed production are independently segregating (this allows for the production of neuters which can account for up to 25% of the offspring), while the phenotypically determined model assumes 50% of the offspring take the form of the mother and 50% take the form of the father. In both cases, all of the variables used, including total pollen and seed production, maximum pollen and seed dispersal distance, sensitivity to inbreeding depression coefficient (δ), and plant density, are directly inputted by the user or derived from user input.

We use exponential decay functions of the form $y = ae^{-bx}$ instead of average or maximum dispersal distances to describe both seed and pollen dispersal. Using exponential decay is consistent with the literature (Cavers 1983, Austerlitz et al. 2004, Klein et al. 2006) and can describe a wide variety of curves, allowing us to model a number of dispersal patterns resulting from various dispersal vectors. We use four such equations, two to describe hermaphrodite and male pollen dispersal and two to describe hermaphrodite and female seed dispersal. The possibility for different dispersal equations between the hermaphrodites and unisexual plants allows for distribution differences, or specialization, one of the proposed pressures in the evolution of dioecy (Freeman et al. 1997). Despite not entering the maximum pollen and seed dispersal distances directly, we can calculate them by determining the value of x for which $y < 1$. At $y = 1$, x is the maximum distance that one pollen grain or seed can travel. Any distance beyond that is outside of the pollen/seed shadow. The total quantity of pollen/seeds produced by a plant can be estimated using this equation and the maximum distance. Starting with $x = 0$, we sum the values of y as x is incremented by one until x = maximum distance. Both maximum distance and total seed/pollen count are constant for a given phenotype.

The total dispersal area is then segmented into concentric rings (the number of rings is inputted by the user) and can range from 1 to the maximum distance - there must be at least one unit of measure between each pair of rings. The proportion of pollen/seeds for each ring is calculated, with the value of the outside radius included in the ring.

Species are not equally sensitive to inbreeding (Lloyd 1979, Richards 2000). Accordingly, we allow users to input a value from 0 to 1, where 0 represents a species that is not affected at all by inbreeding (i.e., selfed individuals are just as fit as outcrossed individuals), and 1 represents a species that is highly affected by inbreeding (i.e., an outcrossed individual is much more fit then a selfed individual). Traditionally, this difference in fitness is measured using a constant inbreeding depression coefficient (δ), where $1-\delta$ is the relative fitness of an individual produced through self-fertilization compared to one produced through outcrossing (Charlesworth and Charlesworth 1978). However, our model considers not only selfed and outcrossed individuals, but also individuals that are the result of varying degrees of incest. Consequently, we replace $1-\delta$ with $1-F\delta$, where δ is inbreeding sensitivity and F is the coefficient of inbreeding of the individual, defined as the chance that two alleles are identical by descent. F theoretically ranges from 0 to 1, but is limited to discrete values obtained using the path method (Ballou 1983). Consequently inbreeding depression in our model is not constant within a population, but changes based on the degree of relatedness between the parents of the individual under consideration.

Another modification is that our model is not a chessboard model, which classically considers all seeds that land in a cell, then randomly chooses the one that lives. In our model the user enters a “circle of death” radius which represents the minimum distance required between two plants for both to survive and reproduce. If, upon dispersal, a seed lands inside another seed's circle of death, competition occurs for that location. The ultimate winner is not chosen randomly; rather the probability of the new seed surviving is based on the relative fitness of both seeds as defined above ($1- F\delta$). Depending on the radius and the number of seeds produced, numerous rounds of competition can occur for a single spot as seeds are distributed. In our model a cell can be thought of as a circle with radius defined by the circle of death; however, these cells are not rigidly located within the garden; they move based on seed location.

Once all initial population information is entered, a loop is run that plants the seeds in the garden with random x and y coordinates. For the initial population, if a seed is planted within the circle of death of another seed, the new seed is simply replanted. If a seed needs to be replanted 500 times with no success then the garden is deemed full.

Each plant is processed separately for pollination. Hermaphrodites and females are considered mothers. Each male whose pollen shadow includes a particular mother (i.e., the distance between the plants is less than or equal to the maximum pollen distance for that phenotype) becomes a potential mate, as does each hermaphrodite whose pollen shadow includes that mother. For each mate the distance between the plants is determined using their (x,y) coordinates, and the amount of pollen that reaches the mother is calculated using the pollen distribution equation for the mate's phenotype. Each mate is recorded as well as the amount of pollen it donates. If excess pollen reaches a mother, we match the number of seeds and pollen by randomly reducing the amount of pollen until it matches the number of potential seeds that phenotype can produce. It is important to note that if the mother is a female, then only other plants can be considered mates, while hermaphrodites can mate with themselves (selfing). At this point, the number and paternity of seeds on each mother is known.

In the genetically determined model, each seed is then given a genotype based on the genotypes of its parents and the assumption of independent assortment. Specifically, there are four possible crosses and associated offspring probabilities: Hermaphrodite x hermaphrodite – results in all offspring have an XX genotype (hermaphrodite). Hermaphrodite x male – results in 50% of the offspring will be X_2X (male), 50% will be XX (hermaphrodite). Female x hermaphrodite – results in 50% of the offspring will be X_1X (female), 50% will be XX (hermaphrodite). Female x male – results in 25% of the offspring will be XX (hermaphrodite), 25% will be X_1X (female), 25% will be X_2X (male), and 25% will be X_1X_2 (neuter).

In the phenotypically determined model, each seed is assigned a phenotype based on the phenotype of its parents. In this case, the four possible crosses and the associated offspring probabilities are: Hermaphrodite x hermaphrodite – results in all offspring are hermaphrodite. Hermaphrodite x male – results in 50% of the offspring will be male, 50% will be hermaphrodite. Female x hermaphrodite – results in 50% of the offspring will be female, 50% will be hermaphrodite. Female x male – results in 50% of the offspring will be female, and 50% will be male.

Seeds are then assigned an F value based on the relatedness of their parents. The initial population is assumed to be outcrossed and assigned an F value of 0.05. This indicates that in a stable, panmictic, hermaphroditic population there is a low level of inbreeding or genetic similarity, which is constant in the population. (The F value 0.05 was obtained using an initial population of outcrossed hermaphrodites (F = 0) and running the program for 100 generations.) The seeds in generation 1 can only be the result of a selfed or outcrossed mating. If the seed is the result of self-fertilization, then it is given an F (coefficient of inbreeding) value of 0.5. If the seed is the result of two non-related hermaphrodites, it is given an F value of 0.05. Otherwise, the seed is considered truly outcrossed and given an F value of 0. For all generations > 1, the following assignments hold: If the seed's mother is also the seed's father, then the seed is selfed and F = 0.5. If the seed's parents are both selfed from the same plant, the seed is a second generation selfed plant and F = 0.75. If one of the seed's parents is selfed and the other parent is outcrossed, then F = 0. If one of the seed's parents is selfed and the other is its half sibling, then F = 0.25. If the seed's parents are siblings from an outcrossed mating, then F = 0.25. If the seed's parents are half siblings from an outcrossed mating, then F = 0.125. Otherwise the seed is considered outcrossed and F = 0.

The F value is calculated using the path method, that is, F = $\Sigma(0.5^{n+1})(1 + F_A)$, where n counts the length of the path relating the seed's father to the seed's mother, and F_A is the correlation of inbreeding of the parent's most recent common ancestor. Therefore, the values above must be multiplied by $(1 + F_A)$ if the most recent ancestor is the product of inbreeding. Theoretically, many more cases than those listed above could be considered, but practically it is not useful to consider cases that result in low F values. The literature suggests that populations will be genetically similar to some extent and that inbreeding must be quite high before detrimental effects are seen (Barrett and Charlesworth 1991; Case 1994; Bartish et al. 1999).

Each seed now has a relative fitness defined as $W = 1 - F\delta$. Individual fitness is first used to determine random seed death or abortion by the mother. For each seed a random number is chosen between 0 and 1. If the random number is greater than W, the seed is aborted. If the random number is less than W, the seed is not aborted.

Seed dispersal follows pollination and fertilization. The dispersal equations and ring count are used to determine the proportion of the total number of seeds in each ring for both hermaphrodites and females. Each mother has a total seed count based on how much pollen reached her and how many seeds she produced. This total seed count is used along with the ring proportions to assign the correct number of seeds to each ring for each of the two mother phenotypes. Upon dispersal, each seed is randomly assigned a radius between its given ring boundaries and an angle θ between 0^0 and 359^0. The equations $x = r\cos\theta$ and $y = r\sin\theta$ are used to convert from r, θ to an x, y coordinate system for placement in the garden. Additionally, since these coordinates are relative to the mother plant, we add the mother's x coordinate to the seed's x coordinate, and the mother's y coordinate to the seed's y coordinate.

Every time a seed is planted inside the garden boundary, a box is created around that seed with a side length equal to the diameter of the circle of death. This box is created to minimize the number of points requiring inspection for potential competition. Each point within this box is examined to see if it is: 1. located in the garden, and 2. occupied by another seed. If both conditions are true, the Pythagorean Theorem is used to determine if the distance between the seeds is less than the diameter of the circle of death. If so, competition occurs to determine which seed remains on the site and which dies. The original seed is called s_1 and variables associated with that seed are given the subscript 1. The new seed is s_2, and variables associated with it are given the subscript 2. The probability of survival of seed 2, $P(s_2)$, is calculated using the algorithm found in Appendix II.

Once $P(s_2)$ is determined, a random number between 0 and 1 is chosen. If the random number is greater than $P(s_2)$, the new seed dies and the original seed retains its spot in the garden. If the random number is less than $P(s_2)$, the new seed lives and the original seed is removed from the garden. A single seed can face many bouts of competition for a site. The same protocol is followed each time.

A set of final counts is made for the surviving plants in the current generation. Counts include the total number of males, females, hermaphrodites, neuters (for the genotype model), and total number of plants. Based on the phenotypes of the surviving plants, the total numbers of X, X_1, and X_2 alleles that survived are calculated. The number of survivors for each allele is compared to the original number that was produced, to obtain the relative fitness of each allele. The protocol and equations used to obtain the relative fitness of each allele are: If alleles are produced but none survive, then the relative fitness is 0. Otherwise, the fitness of X_i is defined as $W(X_i) = \frac{x_i'}{G^0}\frac{G'}{x_i^0}$, where G' = total gametes that survived $(X' + X_1' + X_2')$, and G^o = total gametes produced $(X^0 + X_1^0 + X_2^0)$. The frequency of each allele is then tracked across generations.

Validation

To validate the program we took conditions outlined in Charlesworth and Charlesworth's 1978 model of the evolution of dioecy (Charlesworth and Charlesworth 1978) and attempted to recreate their results using our model. The Charlesworth's model predicts that in a partially self-fertilizing, hermaphroditic population, invasion by a female mutant can occur if:

$$1 + k > 2(1 - s\delta)$$

where k is compensation, (e.g. the extra number of seeds produced by a female compared to a hermaphrodite that are produced as a consequence of not making the androecium), s is the fraction of ovules fertilized by selfing, and δ is the inbreeding depression coefficient. The Charlesworths' second prediction is that invasion by males into the same population can occur if:

$$1 + k > \frac{1 - s\delta}{1 - s}$$

To test these conditions we modified our model by removing incest; i.e., seeds not the product of selfing were considered outcrossed. This eliminates the spectrum of relative fitness values, leaving only w = 1 for outcrossed plants, and w = 1 – δ for selfed plants. Our program does not allow the user to input a number or percent of an individual plant's seeds that result from self-pollination; rather our model operates using a distribution curve, upon which values such as potential number of selfed seeds are based. As a result, in our model a high selfing rate is produced using a steep distribution curve which directly and negatively affects the ability of the population to produce non-selfed seeds in a panmictic fashion.

Despite these challenges unisexual invasion never occurs if the conditions described by the Charlesworth's model are not met. Meeting the conditions, however, does not guarantee invasion. To understand why, a closer look at the equations is helpful. The most stringent conditions occur if there is no compensation by the unisexual plant. Then, the product $s\delta$ must be greater than 0.5. This implies that both s and δ are quite high (~ 0.7). The situation created in our model when selfing rates are high (even 50%) limits pollen available to unisexual plants, not because there is a limited amount of pollen, but because the pollen is not widely distributed (i.e. mating is not panmictic). This limited distribution results in the distinct disadvantage of female unisexual plants not receiving enough pollen to produce a significant number of seeds—even if the seeds produced are more fit. Increasing the population size results in a higher collective pollen distribution and in turn higher seed production, but it also disproportionately increases the number of selfed seeds produced, increasing competition and lowering the odds of unisexual plant survival. Increasing k alleviates the condition's restrictions until both s and δ equal zero, at which point $k > 1$, indicating a unisexual female must at least double the ovule production compared to a hermaphrodite to be able to invade as predicted by Charlesworth and

Charlesworth (1978). Thus, we feel that our model is both valid and fundamentally different from other models and can therefore yield useful insights into the evolution of dioecy.

Simulations

We considered four general simulation conditions for each model type (genetic and phenotypic offspring determination): 1. Compensation effects, 2. Inbreeding effects, 3. Specialization effects, and 4. Interaction effects.

1. Compensation

Compensation is the ability of a unisexual plant to utilize resources saved through the elimination of one sexual function to increase production of the conserved sexual function (Eckhart 1992). To test the role of compensation, all variables are held constant among the phenotypes except *k*, which is given values of 25% and 100% more pollen/seeds than the hermaphrodites.

2. The role of incest and inbreeding

Inbreeding depression is measured in two ways in this program. The primary measure is through sensitivity of the species to inbreeding. Some naturally occurring species propagate completely through self-fertilization (i.e., soybeans, but see Burton and Brownie, 2006), while others experience a severe fitness loss in selfed versus outcrossed offspring (many conifer species) (Eriksson et al., 1973, Karkkainen et al., 1996, Remington and O'Malley, 2000). The second way that inbreeding depression is calculated in this program is through the coefficient of inbreeding (F), which is based upon the degree of relatedness and thus corresponds to incest. The transformation of δ from a constant to a variable based on F is a unique feature of this program. To test the role of inbreeding depression all variables are held constant and inbreeding sensitivity is given values of 0, 0.5, and 0.9. Incestuous relationships are determined based on the criteria outlined earlier in the methods.

3. The role of specialization

Specialization in this model is reflected in the ability of the unisexual phenotype to disperse pollen/seeds farther or differently compared to the hermaphrodite phenotype. Substituting values for the coefficients (a and b) into the equation $y = ae^{-bx}$ results in changes in both the distribution pattern and the number of seeds/pollen distributed. For this test, both coefficients are manipulated so as to keep the number of seeds/pollen relatively stable while increasing the area over which distribution occurs. The four values considered are labeled in terms of the difference between the "b" coefficients of the hermaphrodite and unisexual plants. Additionally we note that although changing the "b" coefficient alters the seed and pollen shadows, it does not do so in an obvious way. A difference of 0.01 in b coefficients is equivalent to a female seed shadow expansion of 7 units compared to a hermaphrodite seed shadow, and a male pollen shadow expansion of 11 units compared to a hermaphrodite pollen shadow. Similarly, a difference of 0.03 in b coefficients is equivalent to a 14 unit expansion for females and a 31 unit expansion for males, a difference of 0.05 is a 36 unit expansion for females and an 80 unit expansion for males, and a difference of 0.07 is a 120 unit expansion for females and a 238 unit expansion for males. Notice that pollen shadows were always augmented more than seed shadows.

4. Interaction effects

We examined four variable combinations: compensation and specialization, specialization and inbreeding, compensation and inbreeding, and compensation, specialization, and inbreeding.

Results

1. Compensation

In both the genetic (Figure 2.1a) and phenotypic (Figure 2.1b) models, unisexual plants did not benefit from any amount of compensation. In all cases they went extinct within eight generations. This holds true for higher compensation values as well.

2. Inbreeding/Incest

Genetic model

If δ = 0, (i.e., there is no fitness advantage to outcrossing), neither the male nor the female phenotype is able to invade the population (Figure 2.2a). As δ increases, males invade and eventually persist in approximately equal proportion to the hermaphrodites (Figure 2.2b). As δ becomes high (≥ 0.9), male invasion still occurs but is then followed by a slow and steady decline in population number, eventually leading to population extinction (Figure 2.2c). If δ is given a value of 0.5 and only the female phenotype is introduced then the number of females in the population slowly increases until females comprise the majority of the population (Figure 3.2d). If we introduce a single male into such a population of females and hermaphrodites (e.g. similar to that in Figure 2d), and if the male phenotype invades, it does so quickly, causing females to decline and allowing hermaphrodites to recover (Figure 2.2e). In some cases, however, the males do not invade, females persist, and a gynodioecious population results. We believe the decline is likely linked to the production of neuters which decreases female fitness.

Phenotypic model

If δ = 0, (i.e., there is no fitness advantage to outcrossing), neither the male nor the female phenotype is able to invade the population (Figure 2.3a). As δ increases, both males and females are able to invade and persist in low numbers while hermaphrodite numbers crash and population extinction often occurs in less than 100 generations (Figure 2.3b and 2.3c). As in the genetic model, if δ is given a value of 0.5 and only the female phenotype is introduced then the number of females in the population slowly increases until females comprise the majority of the population (Figure 2.3d). If a single male is then introduced to this population they invade, the hermaphrodites crash, and then both male and female phenotypes decline and the population goes extinct in all but one run (Figure 2.3e).

3. Specialization

Specialization is measured using the difference in the “b” coefficients of the equations for the different phenotypes. Depending on the value for a, the increase in distance provided by the change in b is variable. For this reason the difference in distance provided by the specialization advantage is displayed on the graphs in Figure 4. Both the genetic and phenotypic models behave similarly; when the unisexuals are given a 0.01 specialization advantage (7 units for females and 11 units for males) they are not able to invade (Figure 2.4a, b). When this advantage is increased to 0.03 (or 14 and 31 units), males invade and persist at low numbers (Figure 2.4c, d). The proportion of the population composed of males increases with increased specialization advantage until at a difference of 0.07 (120 and 238 units) males and hermaphrodites are approximately evenly represented (2.4e, f). Females are never able to invade under increased specialization conditions.

4. Interaction

Genetic Model

Inbreeding/incest and compensation provided results identical to a population exposed only to the inbreeding pressure (Figure 2.5a). There was no interaction effect in this case.

Inbreeding and specialization facilitated male invasion to a higher degree when acting together compared to when each of these pressures acted alone (Figure 2.5b). The rate at which the males are able to invade is not affected, but the proportion of the population composed of male individuals is much higher than in a comparable population where only one of these factors is considered.

Compensation and specialization were looked at under a low compensation condition (a 25% increase for unisexuals) (Figure 2.5c) and a high compensation condition (a 100% increase for unisexuals) (Figure 2.5d). Under low compensation, male invasion does not differ from specialization considered alone. Under high compensation conditions male invasion follows a similar trend but with a higher percent of male individuals in the population compared to when specialization is acting alone.

The combination of all three pressures (inbreeding/incest, compensation, and specialization) allows for both male and female invasion to occur simultaneously (Figure 2.5e). Under low values of inbreeding depression, specialization, and compensation rates, males quickly invade the population and persist at high numbers while females invade and persist at low numbers (< 100 individuals). With inbreeding and compensation held constant, as specialization is increased the female unisexuals are able to persist at higher numbers. Under the conditions $\delta = 0.5$, compensation = 25%, and specialization = 0.05, unisexuals comprise the majority of the population, with males, females, and neuters representing equal proportions of the population and hermaphrodites persisting in low numbers (Figure 2.5f).

Phenotypic Model

Inbreeding/incest and compensation provided results identical to a population exposed only to the inbreeding pressure (Figure 2.6a). There was no interaction effect in this case.

When inbreeding and specialization are examined together, low levels of either inbreeding or specialization result in populations that mimic populations experiencing low levels of inbreeding (Figure 2.6b). As values for both were increased, most runs resulted in a dioecious population. At a specialization value of 0.05 and δ = 0.3, all runs resulted in a dioecious population (Figure 2.6c).

Compensation combined with specialization resulted in populations that behaved similar to those with only the specialization advantage (Figure 2.6d and e).

When inbreeding, specialization, and compensation are combined, dieocious populations evolve in the majority of runs at low values for all three variables (Figure 2.6f). By increasing δ to 0.5, dioecy consistently evolves at very low levels of specialization (0.01) and compensation (25%) in only thirty generations (Figure 2.6g).

Discussion

Dioecy has evolved repeatedly in numerous independent lines (Renner and Ricklefs 1995), yet the forces responsible for this evolution remain unclear. Here, we evaluated the effects of inbreeding, compensation, and specialization singly and in combination. Our results show that dioecy evolves most readily when all three factors are involved – but inbreeding and specialization are more important than compensation. An important difference between the two models we developed is that a male x female mating in the genetic model, which assumes independent segregation, results in ¼ of the offspring being double mutants, or neuters, and ¼ of the offspring being hermaphrodites. The neuters act as a fitness handicap for the unisexuals – neuters are non-reproducing individuals and an evolutionary dead end. The production of ¼ hermaphrodites means that they are constantly being reinserted into the population which prevents a strictly dioecious population from ever evolving. The phenotypic model was created to combat this. Not only are neuters not produced (and thus no selective disadvantage), but hermaphrodites are not continuously being introduced.

Dioecy, as several have argued (Charleworth and Charlesworth 1978, Thomson and Barrett 1981), results in offspring that are not the product of self-fertilization. It does not follow, however, that the offspring (even those produced by unisexuals) are not the result of inbreeding. Incest occurs among relatives and because of the limited distance of pollen and seed dispersal, plants are often found near relatives, allowing for consanguineous matings. Such matings should result in inbreeding depression greater than zero, but less than that which results from selfing. This should be more prevalent among long-lived species because of intergenerational matings, and dioecy is known to be disproportionately common among woody plants (Renner and Ricklefs 1995). Here, we show that incest, in conjunction with specialization and compensation, can lead to a preponderance of unisexual individuals in the population and this happens under less rigorous conditions than when incest is ignored.

Specialization, the ability to enhance pollen and seed dispersal, is more important for the male sexual function than for the female sexual function. In wind pollinated dioecious species, male flowers are often aggregated into catkins that readily mix pollen into air streams and should be much more effective at dispersing pollen greater distances than ancestral hermaphroditic flowers. This should lead to an enhanced pollen shadow and the ability to sire more offspring. In fact, dioecy is strongly associated with abiotic pollination (Renner and Ricklefs 1995) and Freeman et al. (1984) measured the pollen shadows of male and hermaphroditic *Atriplex canescens*, a wind pollinated subdioecious species. On a per unit donor plant volume basis, male plants have a pollen shadow that is significantly larger than that of hermaphrodites. In animal pollinated species, male flowers are often larger than female or hermaphroditic flowers and more attractive to pollinators (Delph et al. 1996, Yakimowski et al. 2011), though we do not know if increasing the attractiveness of flowers enhances the amount of pollen dispersed or the pollen shadow. We have assumed that specialization enhances the pollen shadow. Nevertheless, the adaptations displayed by male flowers clearly show that specialization has played a prominent role in the continual evolution in species with unisexual flowers.

Seed dispersal appears to play less of a role in the evolution of dioecious species than pollen dispersal. Nevertheless, it does enhance the spread of male sterile mutants. We do not want to imply that we believe seed dispersal is not important; biotic seed dispersal is disproportionately common among dioecious species (Renner and Ricklefs 1995) and facilitates colonization. Not surprisingly, dioecious species are more common early in ecological succession—i.e., a condition that favors high seed dispersal compared to climax communities (Wood and Moral 1987).

Thus, our models predict that males can invade under less stringent conditions than females in most cases. It is interesting to note that more species of dioecious plants have a male biased rather than a female biased sex ratio (Sinclair et al. 2011) a result that agrees with our findings. However, our work is in contrast to the conditions outlined in prior theoretical studies, most notably that of Charlesworth and Charlesworth (1978). One of the major differences between our work and that of the Charlesworths is that they assumed pollen was not limiting and mating was panmictic. We, in contrast, have modeled pollen dispersal on an individual basis and have allowed pollen to be finite (and therefore potentially limiting). Our models do not assume that mating is panmictic, but rather that neighbors are more likely to serve as mates, even when pollen distribution covers a large area. As males increase in numbers, the hermaphrodites in the population become more important as seed producers than as pollen producers, and therefore act increasingly as females. (This is especially true if inbreeding depression is significant.) For females to invade under these conditions, they need to out-compete the hermaphrodites as seed producers. If females and hermaphrodites initially produce equal numbers of flowers and fruits, the offspring of females should have the advantage in one-on-one competition with the average seed of a hermaphrodite. However, such competition does not occur in our model - seeds compete one at a time and not on average. Like females, hermaphrodites too produced outcrossed seeds, and thus, the offspring of females do not always have a fitness advantage. Compensation can give females an edge in the production of seeds, but unless the majority of hermaphrodite seeds are the result of selfing it will be difficult for females to invade even with compensation. If females invade a hermaphrodite population before the introduction of males (as in Figures 2d and 3d), the hermaphrodites contribute to the next generation primarily through the male function. Therefore, males must out-compete the hermaphrodites in order to invade. Without specialization or high inbreeding this did not occur. However, with a modest amount of specialization males are able to sire offspring on more mothers than hermaphrodites and thus are able to invade hermaphroditic populations. While we are heartened by our finding of male biases which correspond to what is observed in nature, we also found cases where males and hermaphrodites persist together without females, and yet androdioecy is comparatively rare in nature (Pannell 2002). This may be due to the fact that specialization rarely operates alone and when combined with other factors females are able to invade.

Inbreeding avoidance has often been considered the primary force behind the evolution of dioecy (Lloyd 1973, Charlesworth and Charlesworth 1978). As inbreeding becomes more detrimental to fitness, outcrossed individuals gain an advantage and should outcompete selfed and inbred seeds. Our results in both models suggest that the effect of inbreeding is not straightforward. In the genetic model, increasing the negative effect on fitness through inbreeding allows male individuals to invade and persist in high numbers within a hermaphrodite population, while females are sometimes able to invade but even then, persist only in small numbers. In the phenotypic model, both phenotypes can invade – the males invade quickly then taper out, while female invasion is slow and steady (Figure 2.3b). In both models, if the effect of inbreeding is particularly high (≥ 0.9) and seed dispersal is relatively local, the population tends to go extinct. The graphical display helps explain this result; males invade quickly, outcompeting hermaphrodites, but as males do not produce seeds, their expansion leaves areas devoid of new plants in the next generation due to low seed dispersal. Why females do not invade under these conditions is unclear, but if the male phenotype is not introduced in the initial population, the female phenotype is able to invade at a relatively low inbreeding effect ($\delta = 0.5$). This implies that under the given conditions the female phenotype is more fit than the hermaphrodite. However, if we let the females establish themselves and then introduce the male phenotype, males invade very quickly (< 20 generations in the genetic model) and cause a rapid crash in female numbers (Figure 2.2e). This result suggests that under the given conditions females are less fit than hermaphrodites since, as the males invade, the hermaphrodites become more important as seed producers and are able to function as pollen donors, though less successfully than males. In other words, the presence of the male phenotype lowers the fitness of the female phenotype. The only resource the two phenotypes compete for is space, but why this should affect the females so adversely compared to the hermaphrodites is not obvious but may have much to do with the nonlinear dynamics of the system. In the phenotypic model, when a male is introduced to a gynodioecious population with low dispersal shadows, males invade quickly, hermaphrodites crash quickly, and then males and females slowly decline together.

Finally, it is worth mentioning that in both models under varying, but favorable, conditions, the transition from a hermaphroditic population to a stable dioecious or subdioecious population occurs in approximately thirty generations. The ecological implications of this are that if populations are ephemeral on a time scale less than thirty years, we should never expect dioecy to evolve.

Conclusion

We examined three factors that are thought to play a role in the evolution of dioecy (inbreeding, specialization, and compensation) using genetic and phenotypic offspring determination. Our results suggest that compensation is the weakest of the three pressures considered. Increasing the amount of pollen or seeds produced does not provide enough of an advantage to allow the unisexual to invade. When combined with the other pressures, however, a small amount of compensation (25%) is enough to result in dioecy. Inbreeding depression and specialization proved to be strong selection pressures, especially for the male phenotype. The most important results obtained show the importance of the interaction effect of all three pressures. Through the incorporation of multiple evolutionary pressures dioecy can be obtained under less stringent conditions than previously thought.

CHAPTER 3: WEATHER RELATED MORTALITY AND DECLINE IN ASPEN-DOMINATED FORESTS OF NORTHERN MN, USA

Introduction

Climate variability is a critical driver of ecosystem dynamics (Walther et al. 2002). Many regions across the United States are experiencing increased average temperatures, heavy rainfall, increase in flooding events, or prolonged and/or severe drought (Easterling et al. 2000; U.S. Drought Monitor). These climatic changes amplify disease and insect outbreaks (Raffa et al. 2008), and are also expected to increase extinction rates (McCarty 2001), promote species migration, and alter local plant species composition and ecosystems (Thuiller et al. 2005). In the short term, tree mortality in many forested ecosystems is often associated with extended periods of high temperatures or low annual precipitation, and recent reviews have documented this pattern on a global scale (Allen et al. 2010). As global temperatures continue to rise, erratic and extreme weather events are expected to become both more common and more severe (IPCC 2007). Understanding how forests respond to inter-annual variation in weather is therefore essential for predicting the future impact of changing climate on forest health and sustainability.

Several studies have described the phenomenon of aspen decline and dieback across North America (e.g., Bartos and Campbell 1998; Hogg et al. 2008; Worrall et al. 2008, 2010; Michaelian et al. 2010), most recently characterized by rapid dieback and deterioration of mature aspen without regeneration to replace the mature trees (Frey et al. 2004). Although processes such as infection from insects and disease (Fairweather et al. 2007; Worrall et al. 2010) and succession (Kulakowski 2004; 2006; Kashian et al. 2007) are known to contribute to aspen decline, weather variability is thought by many to be a major but poorly understood contributor to such decline (Frey et al. 2004; Worrall et al. 2010). In a recent study, aspen growth in northwestern Colorado was found to be negatively correlated with temperature, and mortality was linked with consecutive drought events (Hanna and Kulakowski 2012). Few other studies have investigated the relationships between weather variability and aspen growth in detail in ecoregions beyond western North America. More specifically, the explicit relationships between variability and aspen growth and vigor at the stand level – given that aspen decline is a stand-level process – is unknown.

Aspen is a major forest type in the northern Lake States region, where aspen-birch forests account for approximately 26% of the forests with the heaviest concentration of this cover type in northern Minnesota (Miles et al. 1995; Latherberry and Spencer 1996; Schmidt 1997). The extensive coverage of aspen and birch in northern Minnesota is thought to result from frequent wildfires that occurred during the pre-European settlement period. Massive timber harvesting by early settlers and subsequent wildfires have maintained a disturbed landscape which aspen require (Cleland et al. 2001). Aspen mortality has been documented by the Minnesota Department of Natural Resources and the Forest Health Monitoring Program of the US Forest Service (Federal Conditions Report 2007). In 2008, over 150,000 acres (roughly 3% of the total aspen area in Minnesota) of aspen were experiencing decline and affected areas were concentrated in the northeastern part of the state (MN DNR Aerial Survey Report 2011). Aspen forests in Minnesota are highly managed for their value to the paper industry (USDA Forest Service), as well as for their general role in biodiversity and wildlife habitat (Kay 1997).

Observed climate changes in Minnesota between 1900 and 2000 include increased average temperature (annual temperature is increasing at a rate of 0.6°C per 100 years), increased precipitation (average of 67.3 cm from 1941-1970 to 69.6 cm from 1971-2001; NCDC 2002), increased flooding severity, and shorter, warmer winters (the snow season in the Great Lakes region has decreased by 6 days in the last 50 years and average winter temperatures are increasing at 1.3°C per 100 years (NCDC 2002; Ciborowski and Fenske 2003; Seeley 2006). The increase in winter temperature may be especially important because warmer winters result in earlier snow melt and spring transition from snowfall to rainfall. Despite an overall increase in annual precipitation, growing season precipitation has decreased; Minnesota received drought status during the summer months every year from 2003-2008 (U.S. Drought Monitor). As climate change begins to alter ecosystems, trees are generally less affected than annual and herbaceous plants due to their perennial habit, capacity to store nutrients, and ability to resist environmental stress. These traits allow trees to persist through adverse conditions, but their growth rates may be highly responsive to their environment, with precipitation often being the limiting factor (Teskey et al. 1987; Schuur 2003). In this study, we examine how inter-annual variability in average monthly temperature and total annual precipitation affects stand-level annual growth patterns of aspen forests in Minnesota. Examining both healthy and declining stands across northern Minnesota, we asked the following questions:

(1) Are all aspen stands equally responsive to weather variability?
(2) Which abiotic or biotic factors (stand conditions) are correlated with stand responsiveness to climate variability?
(3) Does geography play a role in stand responsiveness to weather variability or in stand vigor?

Methods

Study Area and Field Methods

Aspen stands were selected from three geographical areas in Northern Minnesota: Marcel Experimental Forest (MEF) in Itasca County, Pike Bay Experimental Forest (PBEF) in northern Cass County, and North East Minnesota (NEM) in Itasca, St. Louis, and Lake Counties (Figure

1). Stands from MEF and PBEF were selected from a previous chronosequence study by the U.S. Forest Service (Bradford and Kastendick 2010). Stand ages are based on known clear-cut events; further selection criteria include no management treatments or catastrophic disturbances since the clear-cut, and a stand area of at least 3 ha (Bradford and Kastendick 2010). NEM stands were selected using GIS mapping and identification of declining aspen stands based on work done by the Minnesota Department of Natural Resources. These stands were selected based on the following criteria: the stands were designated as declining − defined by the MN DNR as aspen stands exhibiting combinations of defoliation, discoloration, dieback and/or mortality of aspen individuals (MN DNR Aerial Survey Results 2011), and upon inspection there were no signs of conifer invasion, fungal infection, or insect damage. During June –July 2008 seventeen stands were sampled at PBEF (7 stands) and MEF (5 stands) and classified into one of 5 age categories: 40-60, 60-90, 90-120, and > 120 years. Nine additional stands, all exhibiting decline, were sampled at NEM for a total of 21 stands (Table 3.1). Northern Minnesota exhibits a wide range of forest types including both mixed northern hardwoods and mixed conifer; all the stands sampled were aspen mixed with multiple hardwood and conifer species (Table 3.2).

We used weather data from three different National Oceanic and Atmospheric Administration (NOAA) weather stations, one corresponding to each geographic location. The Detroit Lakes weather station was used for PBEF, the Grand Rapids weather station was used for MEF, and the Duluth weather station was used for NEM. Both Itasca (MEF) and Cass (PBEF) Counties receive an average annual precipitation of between 635 and 762 mm (25-30 inches) and have average annual temperatures of 4.2°C and 5°C respectively (Midwest Regional Climate Center). In the Northeast, St. Louis County receives an average of 635 to 762 mm (25-30 inches) annual precipitation and Lake County receives just over 762 mm (30 inches) average annual precipitation. Average annual temperatures are 3.9°C in St. Louis County and 4.2°C in Lake County.

Within each stand, three 10 meter radius circular plots were randomly established at least 30m from all roads and from each other. Within each plot, all trees with a diameter at breast height (DBH) > 2.5cm were measured and identified to species, mapped using azimuth and distance from plot center, and an increment core sampled at breast height. Rotten trees and stumps were not cored. Between 22 and 118 trees were collected for each stand (average = 70) representing between three and twelve species (average = 7).

Analytical Methods

Increment cores were processed and dated by hand in the lab using conventional techniques (Phipps 1985; Speer 2010). Individual tree chronologies were developed and cross-dated by comparison with other trees within the stand using COFECHA (Holmes 1983), and a master chronology for each stand was produced using the dplR library in R (Bunn 2008; 2010). Stand-level basal area increment (BAI), the amount of wood added annually by the stand, was used as the primary measure of stand growth in this study. Stand-level BAI information was related to monthly total precipitation and average monthly temperature data from the three NOAA weather stations to determine response and correlation functions in DendroClim 2002 (Biondi and Waikul 2004). BAI provides a general measure of stand vigor (as an indicator of stand health), and broad categories of BAI were defined to differentiate among vigor classes. Stands with a decadal average BAI < 5 m^2ha^{-1} were classified as low vigor, while stands with high vigor were considered to be those with an average BAI > 7.5 m^2ha^{-1}. Stands with an average BAI between 5 m^2ha^{-1} and 7.5 m^2ha^{-1} were classified as medium vigor. A previous study reported average BAI of healthy aspen stands to be between 6.62 and 7.11 m^2ha^{-1} in northern lower Michigan (Sakai and Burris 1985), consistent with our classification.

We examined the response of stand BAI to monthly average temperature and precipitation values over both the current and previous year because tree growth may be affected by past conditions (Hanna and Kulakowski 2012). The response coefficients obtained using DendroClim 2002 (coefficients are multivariate estimates from a principal component regression and range from 0 to 1 with higher values accounting for more of the observed variability) characterize the relationship between annual stand-level growth and monthly climate variables (average monthly temperature and total monthly precipitation). We used the moving interval function in which a set interval is progressively moved forward and analyzed 1 year at a time (Biondi and Waikul 2004). Each stand was analyzed individually and an average was calculated for each vigor class. Only significant relationships are reported ($\alpha = 0.05$ using 1000 bootstrapped samples).

To examine stand characteristics associated with high aspen vigor we first performed a set of one-way analysis of variance (ANOVA) to determine if there are any differences in means of 17 stand characteristics across vigor classes. Characteristics considered were: location, aspen quadratic mean diameter (QMD), stand age, oldest aspen tree, total number of trees in the stand, stand density, relative conifer density ((# of conifer stems / # of total stems) x 100), relative aspen density, the relative dominance of conifers ((conifer basal area / total basal area) x 100), maple, birch, oak and aspen, stand basal area, diversity (calculated using the Shannon-Weiner index), and species richness. All variables were tested and met assumptions of normality and heteroscasticity, once diversity, relative density and relative dominance variables were arcsine transformed. Stand age was a categorical variable and was not tested with ANOVA. We then performed a linear discriminant analysis (LDA) in SPSS (2012) using the same 17 stand characteristics to discriminate between vigor classes (Table 3.3). LDA expresses a dependent, categorical variable as a linear combination of a number of other variables. Mean values for each variable within each vigor classification are calculated and used to compare variability among classes. Each function has a corresponding eigenvalue that corresponds directly with the separation of that function. An additional LDA was performed to discriminate between stands that responded to weather variables and those that did not.

Results

Variation in Stand Responsiveness to Average Monthly Temperature

Stands with low vigor responded negatively to previous January (-.026), February (-.029), April (-.028), May (-.095), July (-.036), September (-.058), and current January (-.11), May (-.06) July (-.035), and September (-.035) temperature variables, and had a positive response only to the previous December (.031) temperature variable. Stands with moderate vigor responded negatively to previous July (-.036) temperature and positively to previous February (.039), June (.033), September (.044), and current February (.034), March (.035), April (.094), and September (.098) temperature variables. Stands with high vigor responded negatively to previous June (-.036) temperature and positively to previous March (.035), April (.032), May (.040), and current January (.052), February (.037), and March (.032) temperature variables (Figure 3.2).

Variation in Stand Responsiveness to Total Monthly Precipitation

Stands exhibiting low vigor responded negatively to previous March (-.031), May (-.035), August (-.033), September (-.029), and current May (-.026), and August (-.063) precipitation variables, and had a positive relationship with previous July (.024), October (.032), December (.074), and current January (.035), July (.022), September (.024), and December (.075) precipitation variables. Stands exhibiting medium vigor responded negatively to current January (-.051), February (-.033), and July (-.003) precipitation, and positively to previous May (.054), July (.050), November (.026), and current March (.039), May (.060), September (.035), and October (.037) precipitation variables. Stands with high vigor responded negatively to previous December (-.053) and current August (-.042) precipitation and positively to previous May (.040), June (.033), July (.095), September (.140), October (.193), and current May (.056), June (.083), September (.129), and October (.256) precipitation variables (Figure 3.3).

Variables Affecting Vigor in Aspen

Five of the 17 stand characteristics displayed significantly different means between the vigor classes at $\alpha = 0.1$. QMD ($p = 0.04$), relative aspen density ($p = 0.08$), relative oak dominance ($p = 0.02$), diversity ($p = 0.01$), and species richness ($p = 0.004$) were all important factors in discriminating vigor classes from one another (Table 3.2). The linear discriminant analysis (LDA) performed using these same variables produced discriminant functions with canonical correlations of 0.98 and 0.95, indicating that the functions effectively discriminate between levels of stand vigor, with no overlap and excellent separation in ordinate space (Figure 3.4). LD 1 (x-axis), which explains 72% of the variation, was correlated most strongly with diversity, and secondarily with species richness and relative aspen density. A negative score on the x-axis is therefore indicative of high species richness, while a positive score indicates high relative aspen density and diversity. LD 2 (y-axis) was most strongly correlated with QMD, diversity, and relative aspen density. Negative scores on the y-axis indicate high QMD and relative aspen density, while positive scores indicate high diversity. Low-vigor stands are therefore separated from medium- and high-vigor stands primarily by high species richness. Likewise, high vigor stands have high diversity compared to medium-vigor stands and medium-vigor stands have larger aspen (higher QMD) making up a larger component of the stand (higher relative density; Figure 3.4).

Effects of Geographical Location

Vigor classes were not randomly distributed among geographic location, but were not unique to location either (Table 3.5). PBEF stands were classified as medium and high vigor in almost equal proportion (low = 3, high = 4). NEM stands were almost exclusively low vigor (8 out of 9 stands), but did have one high vigor stand. This coincides with the fact that NEM stands are also declining stands. MEF stands had all three vigor classes represented (low = 1, medium = 2, high = 2).

Aspen stands in the three geographic locations responded differently to temperature (Figure 3.5). PBEF responded positively to five temperature variables: previous May and June (average response = 0.03 and 0.06, respectively), previous September (0.04), current February (0.03), and current September, which was also the strongest response for PBEF (0.08). MEF stands responded positively to five variables (previous and current February (0.05 and 0.06, respectively), previous and current April (0.04 and 0.15), and current March (0.09)) and negatively to three variables (previous and current July (-0.15 and -0.06), and current September (-0.06)). The NEM stands responded positively only to temperatures of the previous December (0.03). All other responses at NEM were negative and include previous (-0.03) and current (-

0.11) January, previous February (-0.03), April (-0.03), and September (-0.06), and previous (-0.1) and current May (-0.06).

Precipitation responses were slightly more consistent between geographical areas (Figure 3.6). PBEF responded positively to seven precipitation variables: previous May (0.08), September (0.09), October (0.09), November (0.02), and current May (0.10), September (0.11), and October (0.10); PBEf responded negatively to previous December (-0.05), current January (-0.04), February (-0.03), and July (-0.04). MEF stands responded positively to previous June (0.04), July (0.22), September (0.04), October (0.05), and current March (0.05), June (0.10), July (0.09), September (0.08) and October (0.14). NEM stands responded positively to five precipitation variables: previous October (0.06), December (0.07), current January (0.03), October (0.04), and December (0.07). Negative responses for NEM stands include previous March (-0.03), May (-0.04), August (-0.03), September (-0.03), and current May (-0.03) and August (-0.09). Thus both PBEF and MEF to responded positively to growing season precipitation (May – October) in both previous and current years, while the NEM stands responded negatively to precipitation in these months, and had positive responses only to late season (October) or winter (December and January) precipitation (Figure 3.6).

Factors Affecting Responsiveness to Climate Variables

Not all stands were responsive to temperature and precipitation variables. Five stands from two locations (NEM = 3 and PBEF = 2) across three vigor classes (low = 2, medium = 1, high = 2) did not respond to any temperature variable and had significantly higher relative maple dominance (F = 5.28, p = 0.03) and stand BAI (F = 3.23, p = 0.09; Table 3.6) than stands that did respond to temperature variables. Stands that did not respond to any precipitation variables (a

NEM stand with low vigor and a PBEF stand with medium vigor) had significantly higher stand density (F = 3.82, p =0.07; Table 3.6) than stands that did respond to precipitation variables.

Discussion

Our results show that the impact of weather variability on the growth of aspen in northern Minnesota depends on the health or vigor of the stand in question. Growth of vigorous aspen stands in northern Minnesota is positively related to temperatures during the late winter and spring seasons and negatively to temperatures from the previous summer. A negative response to warmer growing season temperatures in healthy stands is likely reflective of the current long-term summer drought conditions in Minnesota that cause an increase in evapotranspiration and associated water stress and reduction in vegetative growth (Mogensen 1980). Photosynthesis in aspen during drought periods is also likely to be hindered at high temperatures. Moreover high temperatures may increase maintenance respiration requirements, resulting in less energy available for annual stem growth. The significant (and positive) relationship with winter temperatures in vigorous stands could be a result of the soil's ability to retain winter precipitation through earlier thaw events and an increased frequency of smaller spring runoff events (Dettinger and Cayan 1995), but is more likely related to aspens' ability to photosynthesize through their bark (Foote et al. 1976) and to the production of winter carbohydrates when temperatures in the bark exceed freezing. If the trend of current warming summers and winters continues in this region, aspen growth is likely to experience a tradeoff between increased production of photosynthate during warmer winter months and reduction of growth during droughty summer months, while the increase in spring growth is not likely to compensate for lost growth during the growing season. The redistribution of stand-level aspen growth on an annual basis is a fertile area for future research.

Aspen stands with low vigor (most of which were declining) exhibited a generally negative response to temperature between January and September, in contrast to the responses of high- and medium-vigor stands. The reason for this response is not clear, but is likely explained simply by the generally low growth rate in a low-vigor stand regardless of variability in temperature. Stands in all vigor classes responded similarly to precipitation, with positive responses throughout the growing season. Notably, not all aspen stands classified as “declining” exhibit low vigor, and not all visually healthy stands are vigorous. For example, we identified one stand in NEM − thought to be declining − that had high stand-level BAI, and one seemingly healthy stand that displayed low BAI. Clearly, however, inter-annual variability between temperature and precipitation affects aspen stands differently based on their condition prior to the onset of the weather variability. Stands experiencing disease, herbivory, or intense competition must allocate resources to maintenance, defense, and recovery (Kariyat et al. 2012), leaving fewer resources available for secondary growth.

We also note that specific stand conditions are associated with different vigor levels. Although only five of the measured variables were useful in differentiating stands by vigor class, they were able to separate the groups very clearly using LDA. Stands with the highest diversity and species richness also exhibited the highest vigor (Table 2); diversity indices may be a proxy for site quality, indicating that the most vigorous stands are simply located on the most productive sites. Similarly, stands having high vigor also had the largest trees (QMD). Large trees are preferentially present in the canopy, exposing them to a greater amount of light, and they have a larger root system, allowing them to obtain water resources unavailable to smaller trees. Relative aspen density was highest in low vigor stands, which may also indicate lower site quality where other more nutrient-demanding deciduous species were not present.

Somewhat surprisingly, patterns of stand vigor were not unique to geographical region, although the majority of low-vigor stands were located in northeastern Minnesota. Stands responded differently to temperature and precipitation in each of the three geographical areas, perhaps suggesting that geography drives inter-annual variability in weather that affects aspen vigor. PBEF and MEF are located further inland in a more continental climate than NEM, which is located near the north shore of Lake Superior and likely experiences a more moderate climate. Consistent with our analysis of vigor classes, stands in NEM (8 of 9 having low vigor) responded negatively to temperatures between January and September. Whether the low vigor of declining stands is somehow associated with variations in climate associated with geographical location is an important future research question.

Although weather variability is an important variable influencing growth, we have shown that it is only one of many such variables. Weather alone did not dictate good or bad growing years in the aspen stands studied, and stands did not respond to weather conditions in a uniform way. Future studies that focus on multiple variable effects on growth would help to further address the patterns seen here.

APPENDIX I

Chapter 1 Figures

Figure 1.1: We see that for small *X* (proportion of males in the total population) values, the larger *x* (proportion of males in the progeny of the individual) is the higher the contribution of that individual. Conversely, for large *X* values, a small *x* value gives a higher *C*. As the total sex ratio approaches unity, (proportion of males = ½), the proportional difference on an individual basis loses importance and disappears entirely at *X* = ½ (Shaw and Mohler, 1953).

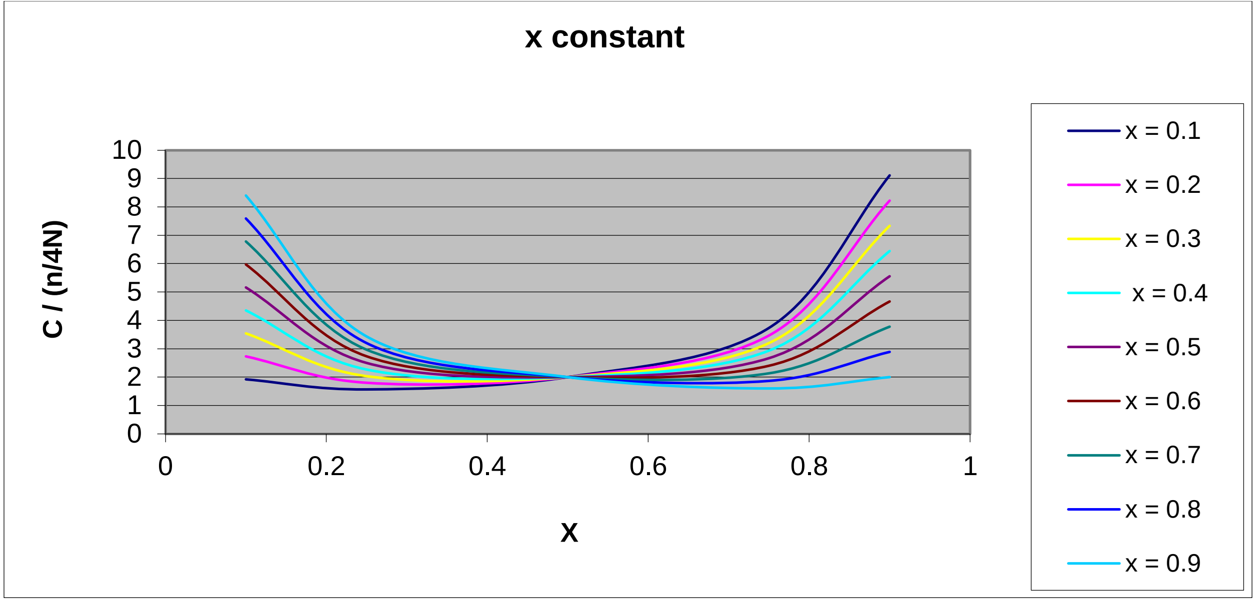
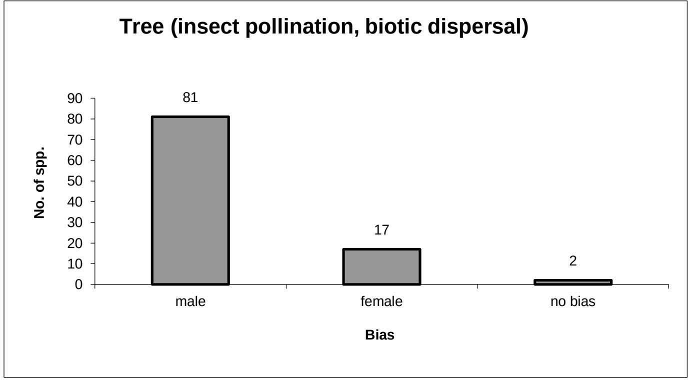
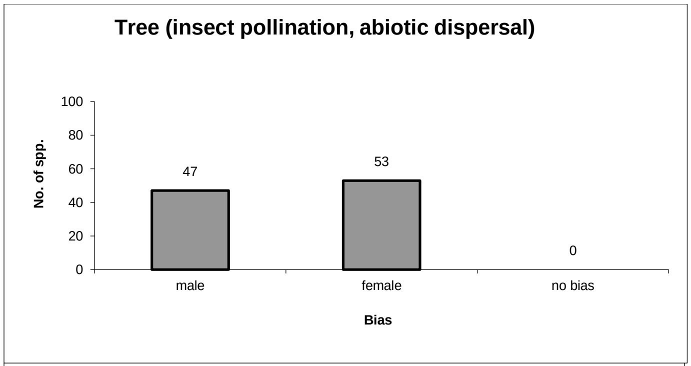
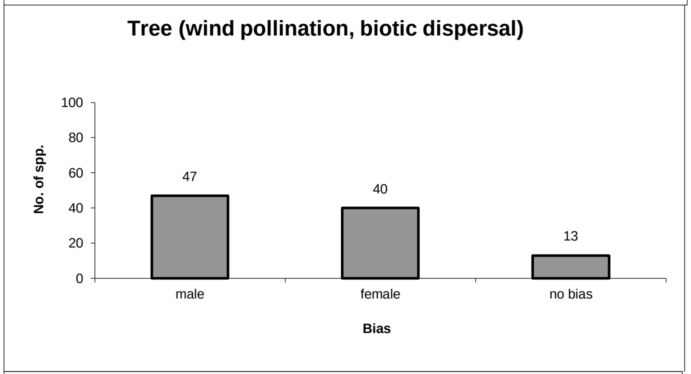
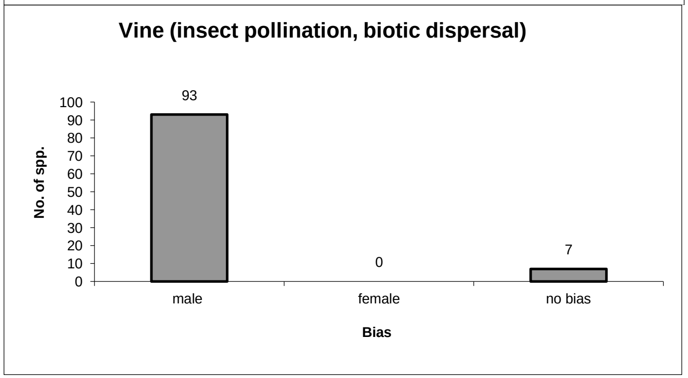


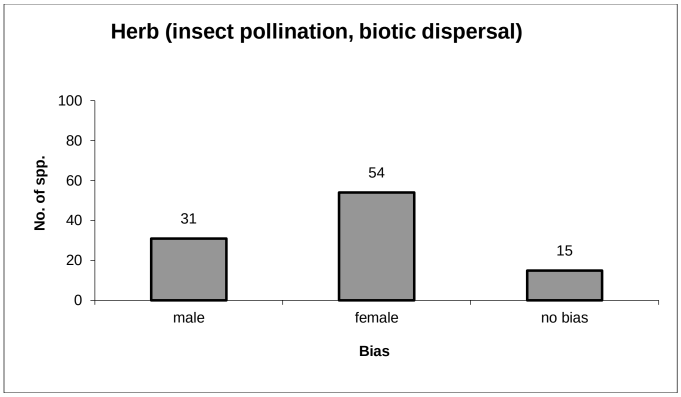
Figure 1.2: Graphical representations of select categories from Table 5 showing the percentage of species in each category displaying either male, female or no bias.

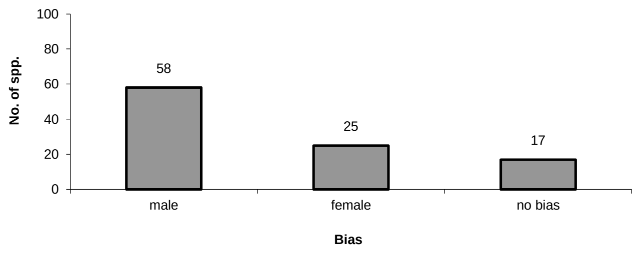


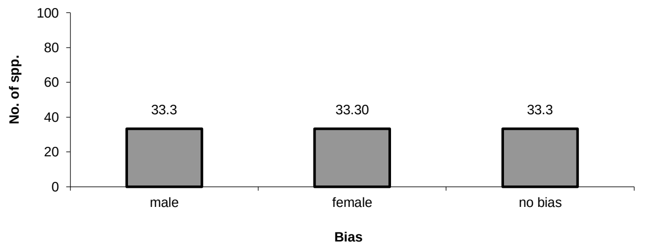

Tree (insect pollination, abiotic dispersal)
No. of spp.
100
80
60
40
20
0
47
53
0
male
female
no bias
Bias

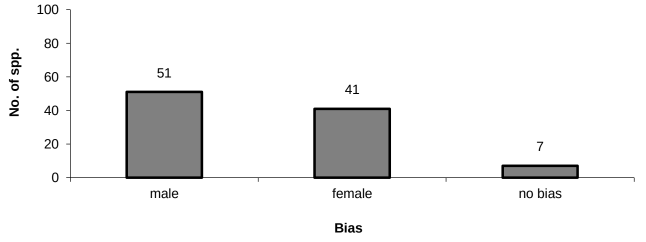

Tree (wind pollination, biotic dispersal)
No. of spp.
100
80
60
40
20
0
47
40
13
male
female
no bias
Bias

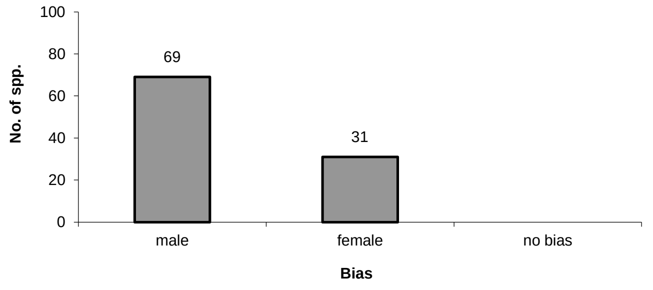

Vine (insect pollination, biotic dispersal)
No. of spp.
100
90
80
70
60
50
40
30
20
10
0
93
0
7
male
female
no bias
Bias

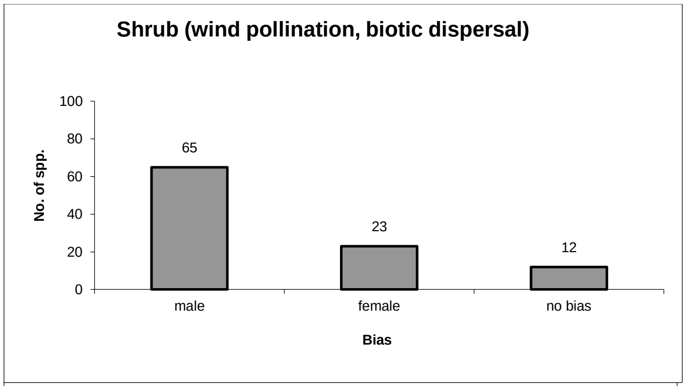

Herb (insect pollination, biotic dispersal)
No. of spp.
100
80
60
40
20
0
31
54
15
male
female
no bias
Bias

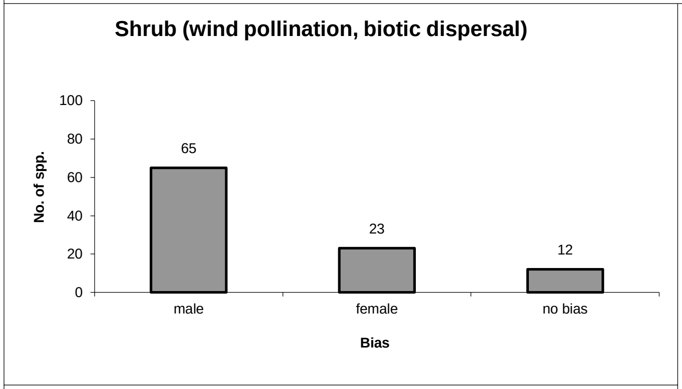

Herb (insect pollination, abiotic dispersal)


Herb (wind pollination, abiotic dispersal)


Shrub (Insect pollination, biotic dispersal)


Shrub (insect pollination, abiotic dispersal)


Shrub (wind pollination, biotic dispersal)


Shrub (wind pollination, biotic dispersal)

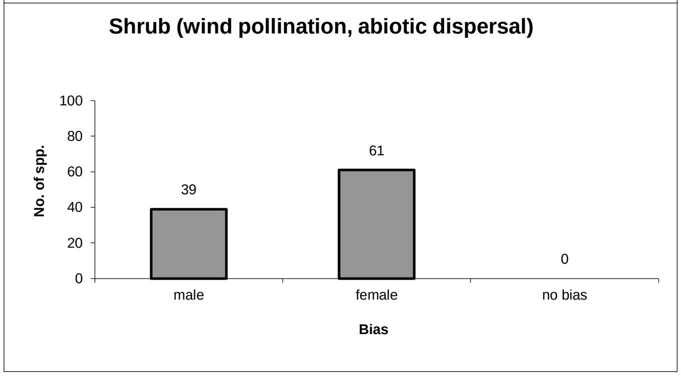

Shrub (wind pollination, abiotic dispersal)

Table 1.1: A family level comparison between the number and type of dioecious species that have been studied and those that exist.

Distribution

	all dioecy	studied
tropical	145 (130.6)	15 (29.4)
temperate	77 (91.4)	35 (20.6)

P = 0.05
Df = 1
c.v = 3.84
X^2 = 20.98

Life form

	all dioecy	studied
tree	85 (96.9)	38 (26.1)
herb	70 (70.9)	20 (19.1)
shrub	98 (85.1)	10 (22.9)

P = 0.05
Df = 2
c.v = 5.99
X^2 =16.16

pollination

	all dioecy	studied
abiotic	53 (62.1)	21 (11.9)
biotic	124 (114.9)	13 (22.1)

P = 0.05
Df = 1
c.v = 3.84
X^2 = 12.76

Table 1.2: Evidence for differential resource allocation to reproduction between the sexes. Values obtained from: * Queenborough et al. 2007, ** Delph 1990, *** Gross and Soule 1981, and ^ Wallace and Rundel 1979. Female estimates for both *** and ^ are at 100% seed set.

species	**male flower mass (g/m^2)**	**female fruit mass (g/m^2)**	**ratio(f/m)**
Otoba glycycarpa*	3.6	101.3	28.14
Virola duckei*	4	40.6	10.15
Virola pavonis*	5.61	64.9	11.57
Virola obovata*	1.61	12.9	8.01
Virola cf. calophylla*	3.05	94.7	31.05
	male mg/fruit	**female mg/fruit**	
Hebe subalpina**	129.5	248.8	1.92
	% of wieght in male reproductive structures	**% of wieght in female reproductive structures**	
Silene alba***	47.4	80	1.69
Simmondsia chinensis^	10-15	30-40	2.80

Table 1.3: Number of male and female spinach plants grown under wet vs. dry conditions. Data is taken from Freeman and Vitale, 1985.

Experiment	**Male Plants**	**Female Plants**
Pop. 1: Dry week 1	73	33
Pop. 1: Dry week 2	67	30
Pop. 1: Dry week 3	57	31
Pop. 1: Wet week 1	47	46
Pop. 1: Wet week 2	37	46
Pop. 1: Wet week 3	33	40
Pop. 2: Dry week 1	99	0
Pop. 2: Dry week 3	155	34
Pop. 2: Dry week 5	143	75
Pop. 2: Wet week 1	74	0
Pop. 2: Wet week 3	138	76
Pop. 2: Wet week 5	117	94

Table 1.4: A subset of species sorted according to life form, dispersal agent, pollinating agent, and sex ratio.

Family	**Genus**	**Species**	**bias**	**pollinator**	**lifeform**	**disperser**
Polygonaceae	Rumex	acetosa	f	animal	herb	wind
Poaceae	Distichlis	spicata	f	wind	herb	water
Araceae	Arisaema	triphyllum	m	insect	herb	bird
Chenopodiaceae	Spinacia	oleraceae	m	wind	herb	gravity
Colchicaceae	Wurmbea	dioica	m	insect	herb	wind
Liliaceae	Asparagus	palaestina	none	insect	herb	gravity
Salicaceae	Salix	lanata	f	insect	shrub	wind
Chenopodiaceae	Atriplex	cuneata	m	wind	shrub	gravity
Myristicaceae	Myrica	gale	m	wind	shrub	bird
Moraceae	Ficus	carica	none	insect	shrub	animal
Lauraceae	Lindera	benzoin	none	insect	shrub	bird
Sapindaceae	Acer	negundo	f	wind	tree	wind
Fagaceae	Quercus	gambelii	m	wind	tree	animal
Lauraceae	Laurus	azorica	m	insect	tree	bird
Moraceae	Chlorophora	tinctoria	m	wind	tree	bird
Arecaceae	Borassus	aethiopum	m	insect	tree	animal
Arecaceae	lodoicea	maldivica	m	insect	tree	gravity
Cucurbitaceae	Cucurbita	moschata	m	insect	vine	animal
Dioscoriaceae	Tamus	communis	none	wind	vine	bird

Table 1.5: Categorical breakdown and cell frequencies of 250 dioecious species taken from the literature and used in the log-linear analysis.

		Biotic Dispersal			**Abiotic Dispersal**		
Lifeform	**Pollinator**	**Male bias**	**female bias**	**no bias**	**Male bias**	**female bias**	**no bias**
shrub	insect	15	12	2	11	5	0
	animal	1	0	0	0	0	0
	wind	11	4	2	7	11	0
herb	insect	4	7	2	14	6	4
	animal	1	1	0	0	1	0
	wind	2	3	0	5	5	5
tree	insect	43	9	1	8	9	0
	animal	0	0	0	0	0	0
	wind	7	6	2	2	1	2
vine	insect	13	0	1	4	0	0
	animal	0	0	0	0	0	0
	wind	0	0	0	0	1	0

Table 1.6: Pollen and seed dispersal agents and their corresponding peak (or average), and maximum distances.

Pollen Dispersal

type	description	peak dist.(m)	max dist.(m)	reference
wind	herb (tall)	<3	42	(Lavigne et al., 1998)
wind	herb (short)	0.2	1.2	(Tonsor, 1985)
wind	tree	65		(Sork et al., 2002)
wind	conifer tree	<11	600	(Robledo-Arnuncio and Gil, 2004)
wind	tree	85	2000	(Bittencourt and Sebbenn, 2007)
insect	tropical tree	ave = 210	>300	(Stacy et al., 1996)
butterfly	herb	<2	31	(Webb and Bawa, 1983)
hummingbird	shrub	<10	225	(Webb and Bawa, 1983)
bumblebee	herb	5.9	<200	(Widén and Widén, 1990)
african honey bee	tropical tree		3200	(Dick, 2003)

Seed Dispersal

type	description	peak dist.(m)	max dist.(m)	reference
wind	32m tree (fruit)	10		(Augspurger and Hogan, 1983)
wind	tree (seed)	<5	101	(Clark et al., 2005)
bird	small (tropical)	<100		(Westcott and Graham, 2000)
bird	small	<51		(Jordano et al., 2007)
bird	medium	110		(Jordano et al., 2007)
bird	medium	<5	473	(Clark et al., 2005)
bird	tree	average = 83	291	(Bittencourt and Sebbenn, 2007)
mammal	tree	495		(Jordano et al., 2007)
animal	monkey	5-60	90	(Clark et al., 2005)
ant	herb (temperate)		35	(Cain et al., 1998)

APPENDIX II

Determining survival between competing seeds

The probability of survival of a seed landing within another seed's circle of death is based on the fitness of both seeds. Fitness (w) is relative to the degree of relatedness of the seeds parents (F) in addition to inbreeding depression sensitivity of the population (δ). The algorithm to determine the probability of the second seed surviving ($P(s_2)$) is outlined below:

If $\delta = 1$ and $F_1 = F_2$ then $P(s_2) = 0.5$. If $\delta = 1$ and $F_1 > F_2$ then F_2 lives and F_1 dies. If $\delta = 1$ and $F_1 < F_2$ then F_1 lives and F_2 dies.

If $\delta = 0$ then $P(s_2) = 0.5$ for all F values.

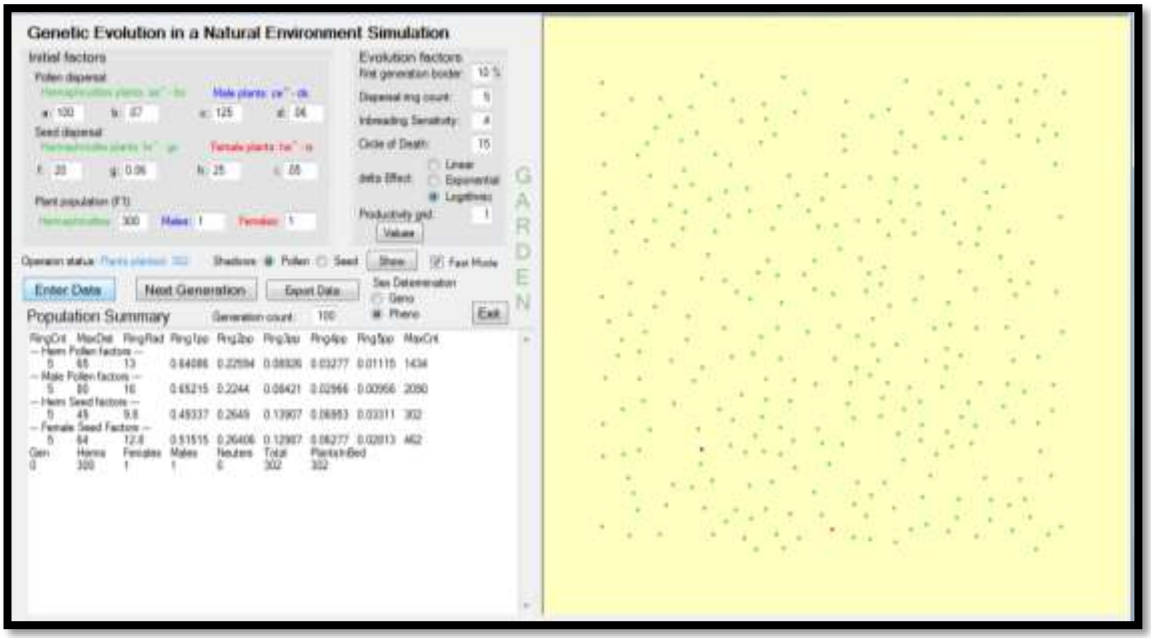
For $0 < \delta < 1$, a loop is run which determines $P(s_2)$. We first define $\Delta = F_1 - F_2$. If $\Delta = 0$, $P(s_2) = 0.5$. Otherwise, we define $w_1 = 1 - F_1\sigma$, $w_2 = 1 - F_2\sigma$, and $d_w = w_1 - w_2$. Then $P(s_2) = 0.042 * \log(d_w) + 0.97$. This is a logarithmic equation that increases with a positive d_w, up to a 97% chance of survival if s_2 is outcrossed and s_1 is selfed.

Linear and exponential equations were used instead of the logarithmic equation to determine $P(s_2)$ in various runs. In both cases unisexuals were rarely able to invade.

APPENDIX III

Chapter 2 Figures

Image 1: Initial population output for hermaphrodites (pollen and seed), males (pollen), and females (seed). Information includes: ring count (RingCnt), the maximum distance travelled by pollen or seed (MaxDist), ring radius (RingRad), the proportion of pollen/seeds in each ring, and the maximum number of pollen/seeds produced (MaxCnt).



a: 100 b: .07 c: 125 d: .06
f: 20 g: 0.06 h: 25 i: .05
Hermaphrodites: 300 Males: 1 Females: 1
First generation border: 10 %
Dispersal ring count: 5
Inbreeding Sensitivity: .4
Circle of Death: 15
delta Effect: ○ Linear ○ Exponential ● Logarithmic
Productivity grid: 1
Operation status: Plants planted: 302
Shadows: ● Pollen ○ Seed
☑ Fast Mode
Sex Determination: ○ Geno ● Pheno
Generation count: 100

```
RingCnt  MaxDist  RingRad  Ring1pp  Ring2pp  Ring3pp  Ring4pp  Ring5pp  MaxCnt
-- Herm Pollen factors --
   5      65       13      0.64086  0.22594  0.08926  0.03277  0.01115  1434
-- Male Pollen factors --
   5      80       16      0.65215  0.2244   0.08421  0.02966  0.00956  2090
-- Herm Seed factors --
   5      45       9.8     0.49337  0.2649   0.13907  0.06953  0.03311  302
-- Female Seed Factors --
   5      64       12.8    0.51515  0.26406  0.12987  0.06277  0.02813  462
Gen      Herms    Females  Males    Neuters  Total    PlantsInBed
0        300      1        1        0        302      302
```

Figure 2.1a: Genetic model results. Low and high (25% and 100%) compensation rates with no specialization or inbreeding depression advantages for the unisexual plants.

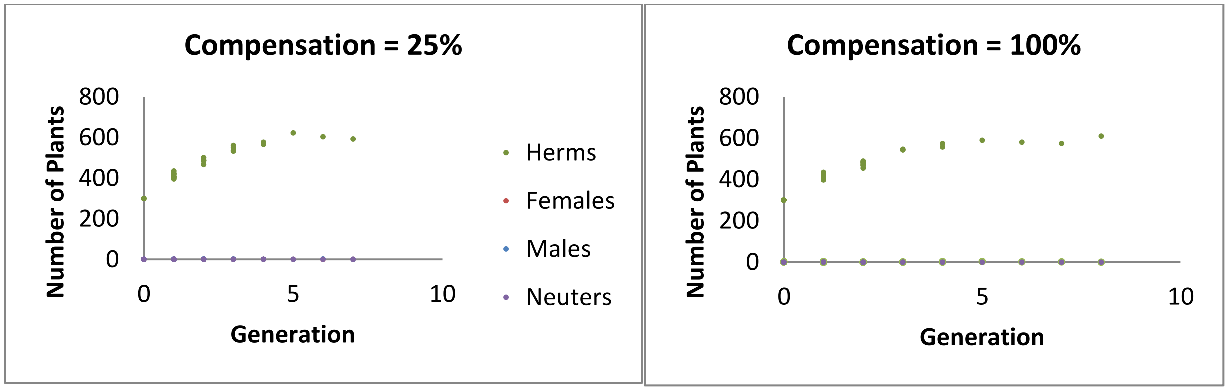


Figure 2.1b: Phenotypic model results. Low and high (25% and 100%) compensation rates with no specialization or inbreeding depression advantages for the unisexual plants.

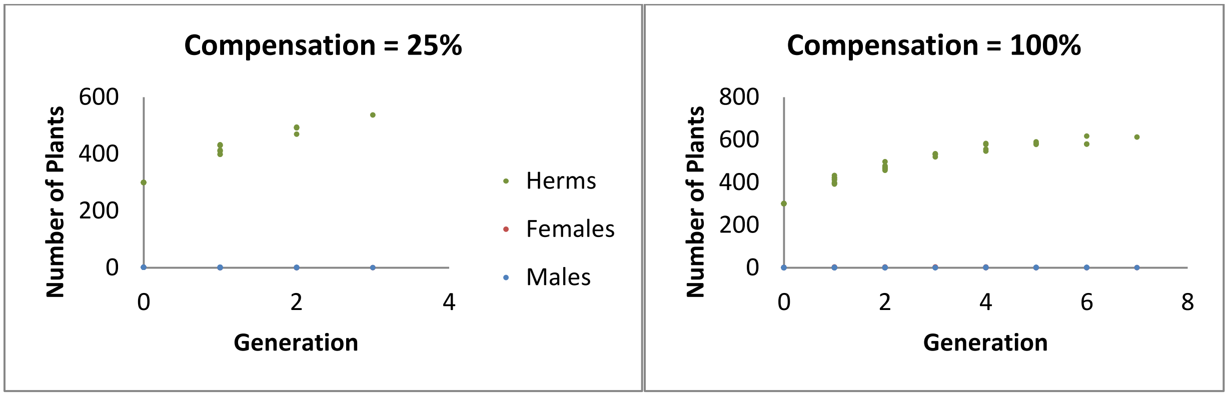


Figure 2.2: Genetic model results. No specialization or compensation advantage.

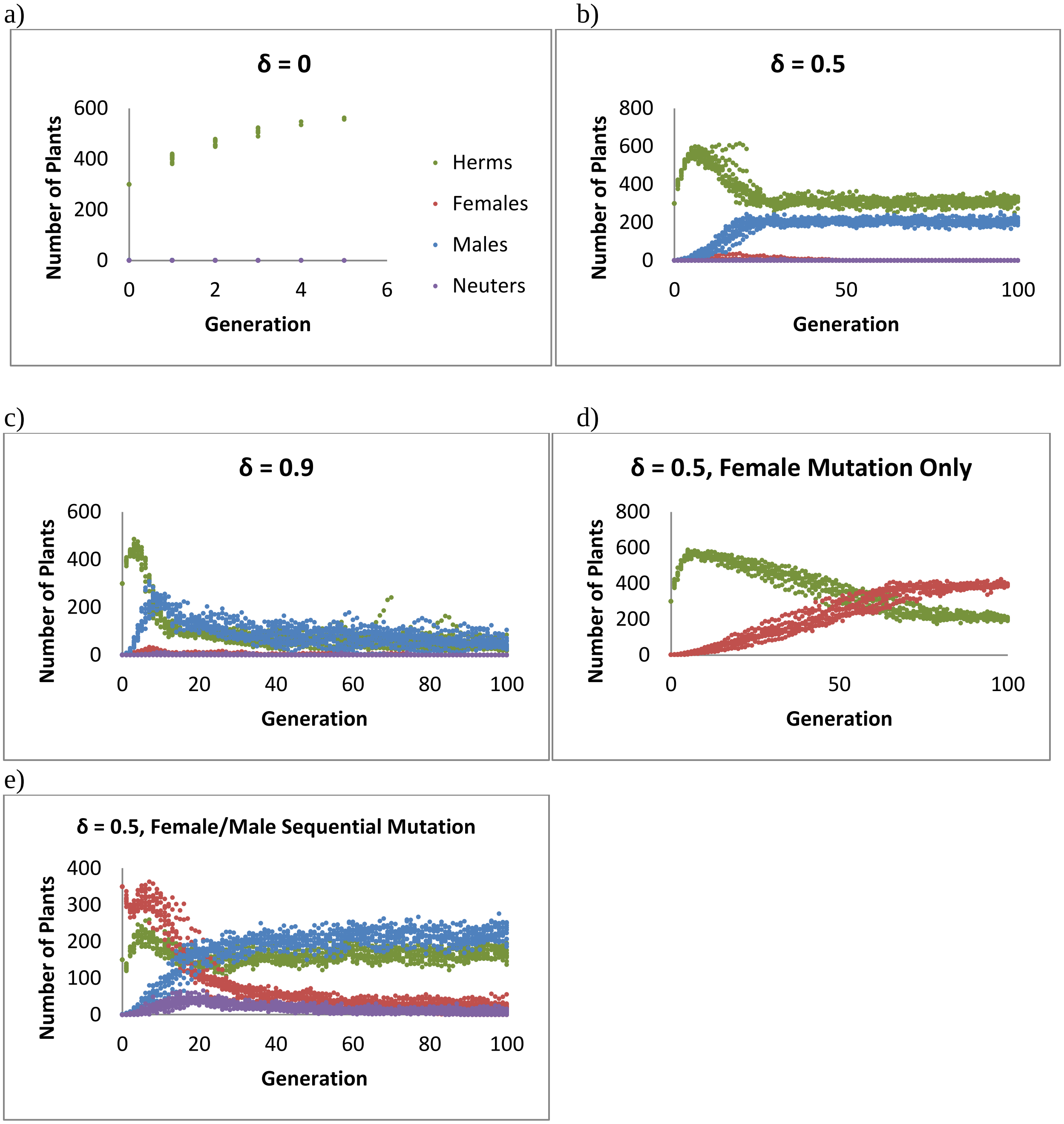


Figure 2.3: Phenotypic model results. No specialization or compensation advantage

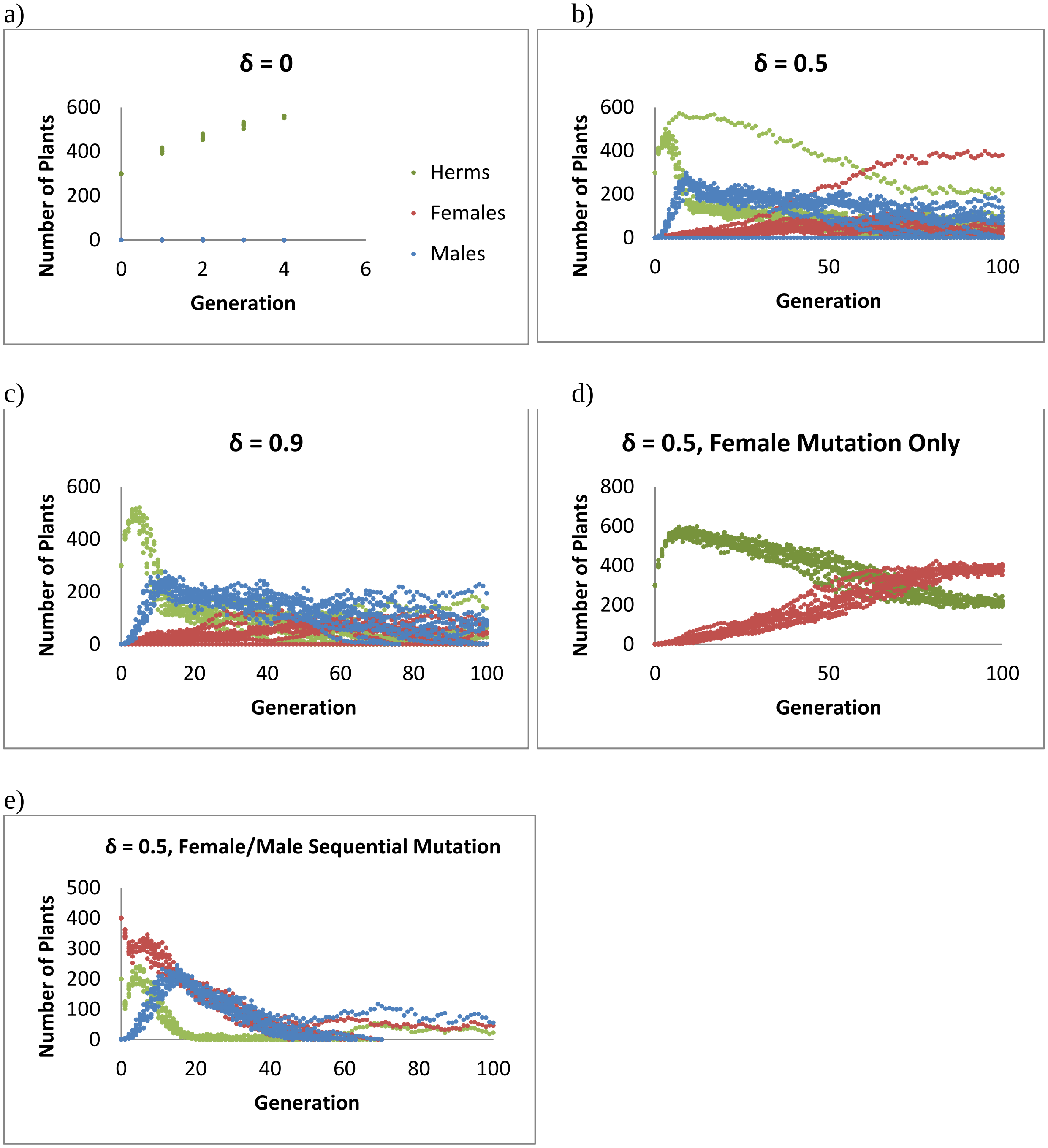
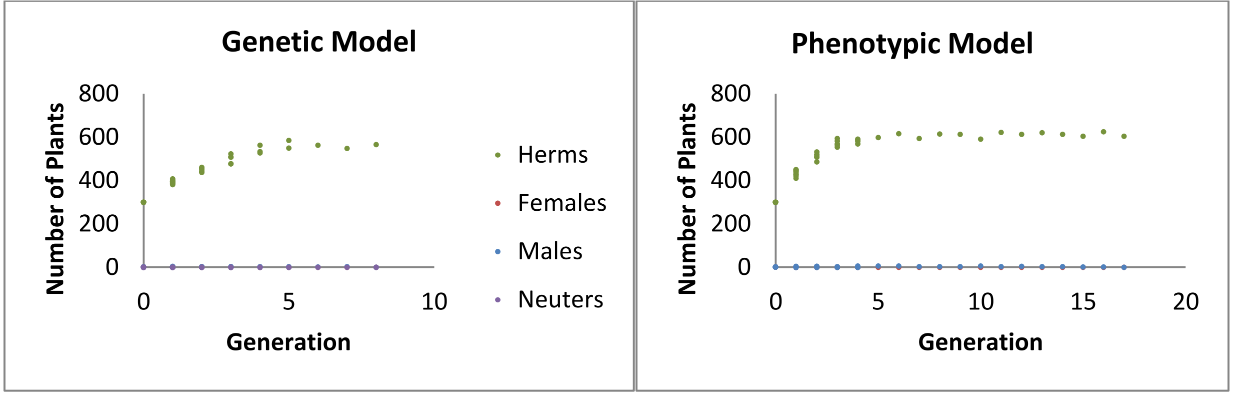
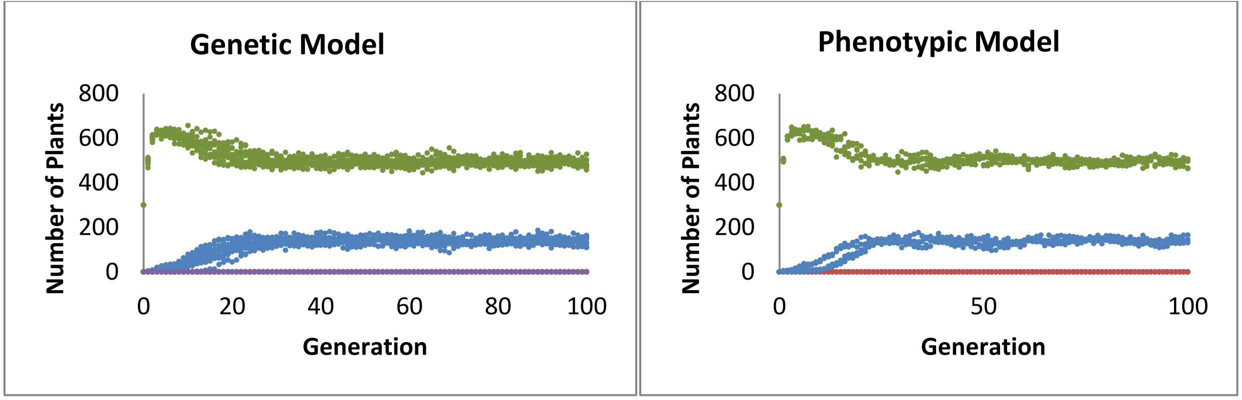


Figure 2.4: No inbreeding or compensation advantages.

a) and b) Specialization = 0.01 (f: 10 units, m: 11 units)



c) and d) Specialization = 0.03 (f: 14 units, m: 31 units)



e) and f) Specialization = 0.07 (f: 120 units, m: 238 units)

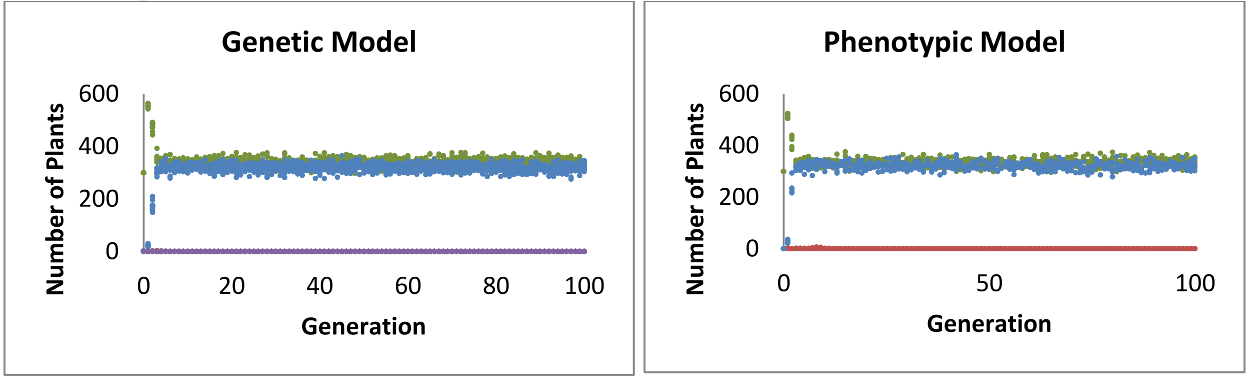
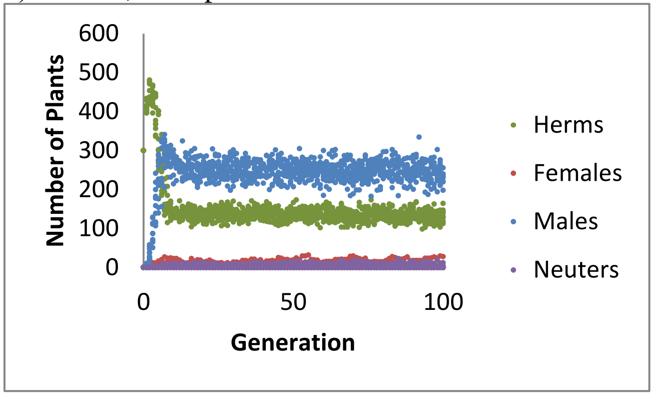
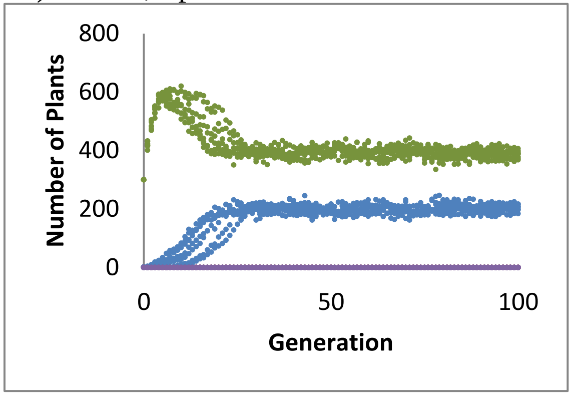


Figure 2.5: Genetic model interaction results

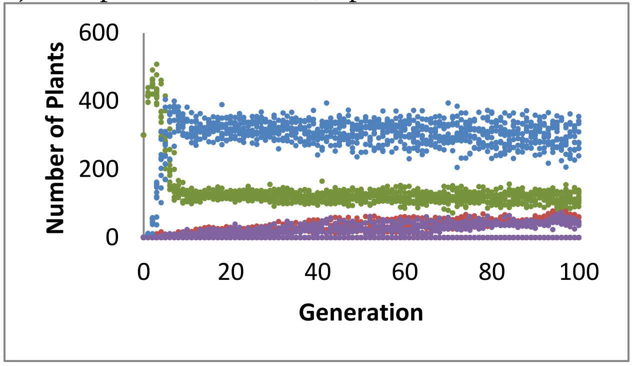
a) δ = 0.5, Compensation = 100%



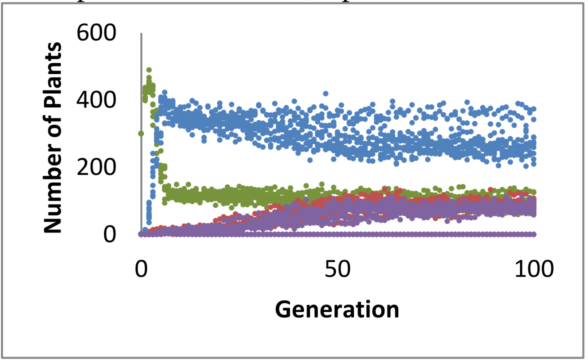
b) δ = 0.5, Specialization = 0.05



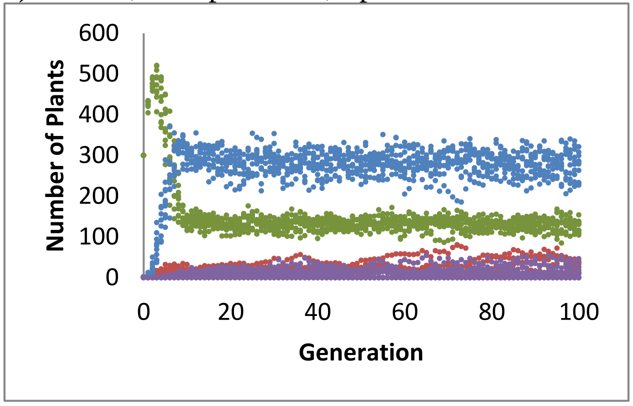
c) Compensation = 25%, Specialization = 0.03



d) Compensation = 100%, Specialization = 0.03



e) δ = 0.5, Comp. = 25%, Sp. = 0.01



f) δ = 0.5, Comp. = 25%, Sp. = 0.05

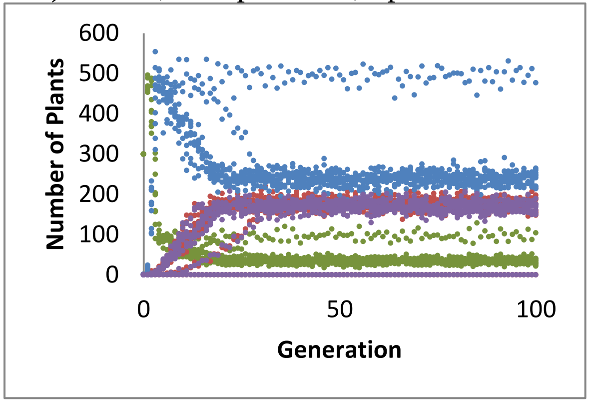
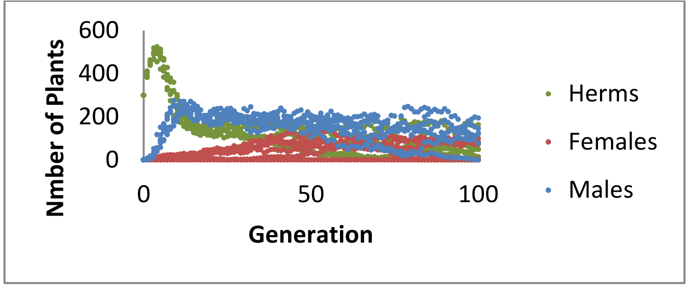
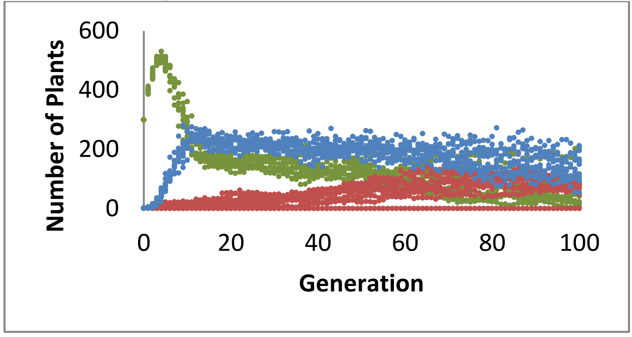


Figure 2.6: Phenotipic model interaction results

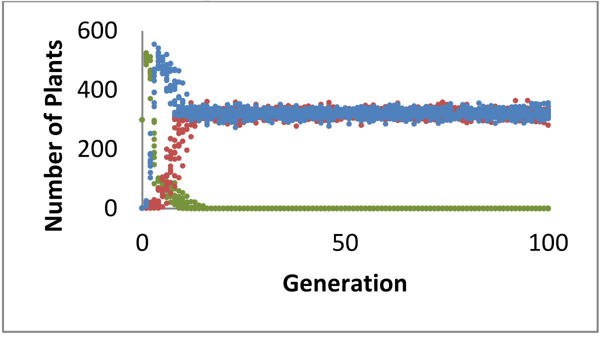
a) δ = 0.5, Compensation = 100%



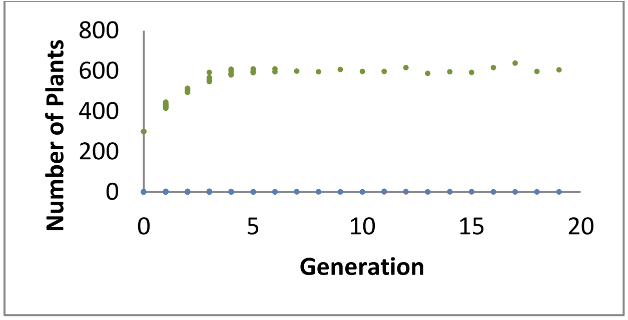
b) δ = 0.3, Specialization = 0.01



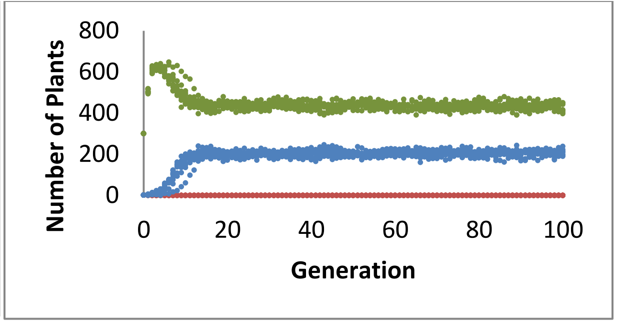
c) δ = 0.3, Specialization = 0.05



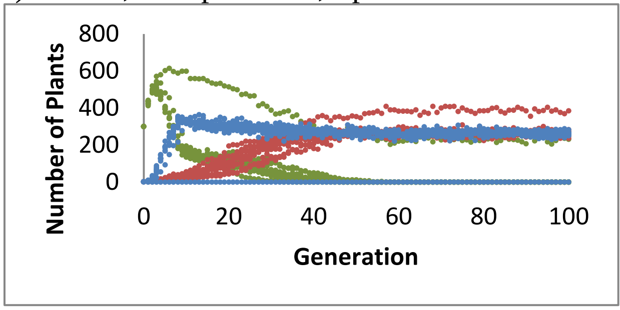
d) Compensation = 100%, Specialization = 0.01



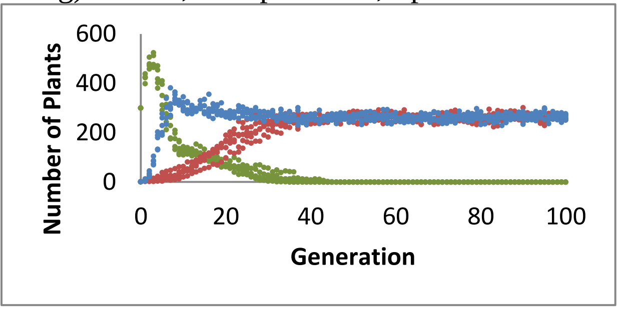
e) Compensation = 100%, Specialization = 0.03



f) δ = 0.3, Comp. = 25%, Sp. = 0.01



g) δ = 0.5, Comp. = 25%, Sp. = 0.01



APPENDIX IV

Chapter 3 Figures

Figure 3.1: Study sites at three locations in Northern Minnesota

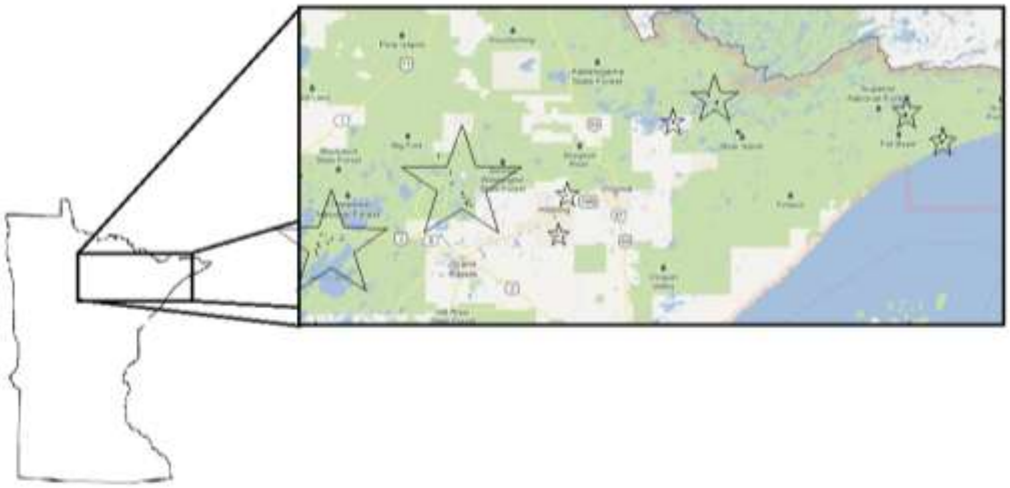


Table 3.1: Age classification of 21 sample sites at three geographic locations in northern Minnesota. MEF = Marcell Experimental Forest, NEM = northeast Minnesota, and PBEF = Pike Bay Experimental Forest.

Location	Site	Age Class	Location	Site	Age Class	Location	Site	Age Class
PBEF	1	60-90	MEF	1	>120	NEM	1	60-90
	2	90-120		2	90-120		2	60-90
	3	60-90		3	60-90		3	90-120
	4	60-90		4	60-90		4	90-120
	5	40-60		5	40-60		5	90-120
	6	20-40					6	60-90
	7	20-40					7	>120
							8	90-120
							9	40-60

Table 3.2: Characteristics for each of the 21 aspen stands sampled in northern Minnesota.

Location	Stand	Stand Density (trees/ha)	Proportion Aspen	Stand Basal Area (m^2/ha)	Aspen	Conifer	Oak	Maple	Birch
PBEF	1	636.62	0.13	170.97	0.12	0.02	0.08	0.83	0.01
	2	880.66	0.12	32.96	0.34	0.05	0.20	0.60	0.04
	3	413.80	0.41	25.43	0.82	0.03	0.21	0.03	0.03
	4	721.50	0.29	48.22	0.53	0.09	0.16	0.21	0.06
	5	859.44	0.77	34.14	0.87	0.03	0.00	0.00	0.07
	6	1230.80	0.34	28.38	0.41	0.00	0.19	0.05	0.14
	7	1029.20	0.73	34.41	0.88	0.00	0.10	0.02	0.00
NEM	1	594.18	0.50	22.76	0.77	0.12	0.00	0.06	0.00
	2	838.22	0.75	18.08	0.74	0.09	0.00	0.17	0.00
	3	488.08	0.89	11.40	0.96	0.00	0.00	0.05	0.03
	4	827.61	0.91	15.26	0.92	0.05	0.00	0.00	0.04
	5	1167.14	0.48	37.24	0.72	0.10	0.00	0.09	0.08
	6	488.08	0.37	16.08	0.44	0.10	0.00	0.00	0.45
	7	562.35	0.55	34.14	0.69	0.23	0.00	0.00	0.08
	8	594.18	0.36	19.60	0.62	0.00	0.06	0.12	0.10
	9	700.28	0.77	10.61	0.68	0.06	0.00	0.00	0.02
MEF	1	594.18	0.36	41.88	0.51	0.40	0.00	0.02	0.02
	2	880.66	0.08	28.87	0.68	0.06	0.05	0.19	0.06
	3	817.00	0.26	38.32	0.70	0.01	0.00	0.27	0.00
	4	233.43	0.23	15.90	0.53	0.00	0.00	0.06	0.14
	5	657.84	0.58	24.29	0.75	0.00	0.00	0.11	0.01

Table 3.3: Average values (with F-statistic and corresponding p-values) of 17 characteristics used to describe aspen stands of low (n = 9), medium (n = 5), and high (n = 7) vigor in northern Minnesota. Diversity, relative density, and relative dominance data are arcsine transformed. * indicates significant difference between classification means (α = 0.1).

	Aspen Vigor				
Variable	**Low**	**Medium**	**High**	**F**	**p**
Location	2.1111	1.8	1.7143		
Relative BAI	0.0232	0.0224	0.0271	0.156	0.857
QMD	22.4111	28.86	33.8571	4.016	0.036*
Age	2.3333	1.8	2.1429		
Oldest Tree	76.3333	84.6	76.5714	0.159	0.854
Total Trees	35.8889	25	32.7143	0.462	0.638
Stand Density	723.8603	874.2912	618.4306	1.731	0.205
Relative Conifer Density	0.2714	0.192	0.2588	0.25	0.782
Relative Aspen Density	0.8889	0.5433	0.733	2.918	0.08*
Relative Conifer Dominance	0.2099	0.1657	0.2454	0.279	0.76
Relative Maple Dominance	0.2356	0.479	0.2717	1.22	0.318
Relative Birch Dominance	0.2138	0.2371	0.2068	0.049	0.953
Relative Oak Dominance	0.0067	0.1193	0.0563	4.95	0.019*
Relative Aspen Dominance	1.0427	0.8319	0.928	1.388	0.275
Stand BA	21.0373	32.5465	50.9794	1.733	0.205
Diversity	0.7087	1.0455	0.95	5.42	0.014*
Species Richness	5.5556	9.6	8	7.421	0.004*

Figure 3.2: Average stand responsiveness to monthly temperature over the current and previous year for each of the three vigor classifications (low, medium, and high) in northern Minnesota.

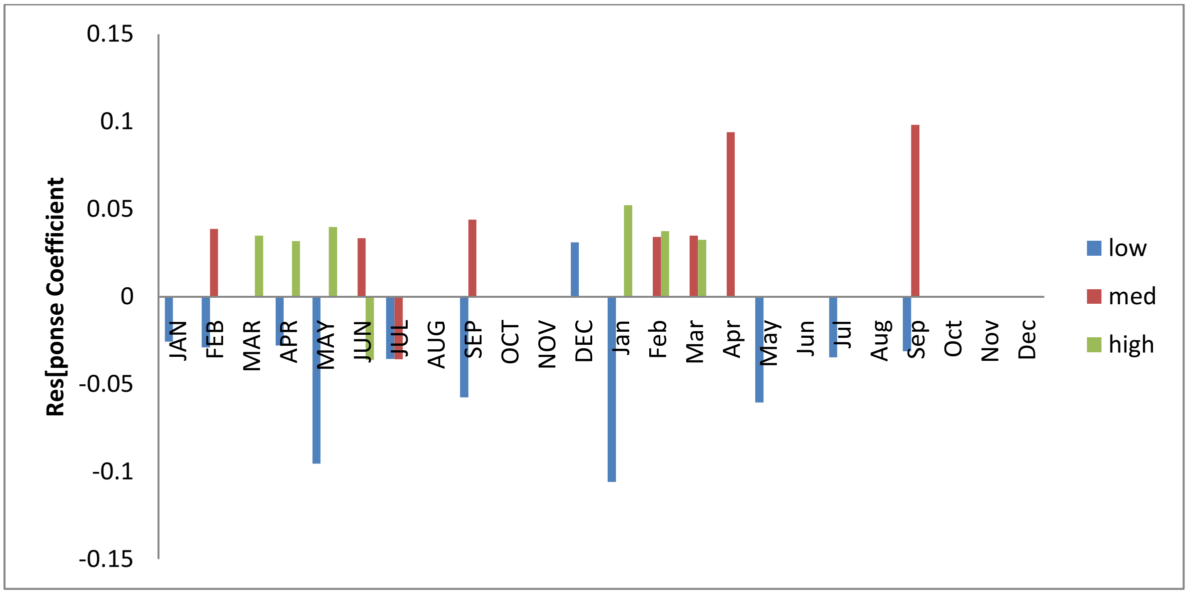


Figure 3.3: Average responsiveness to monthly precipitation over the current and previous year for each of the three vigor classifications (low, medium, high) in northern Minnesota.

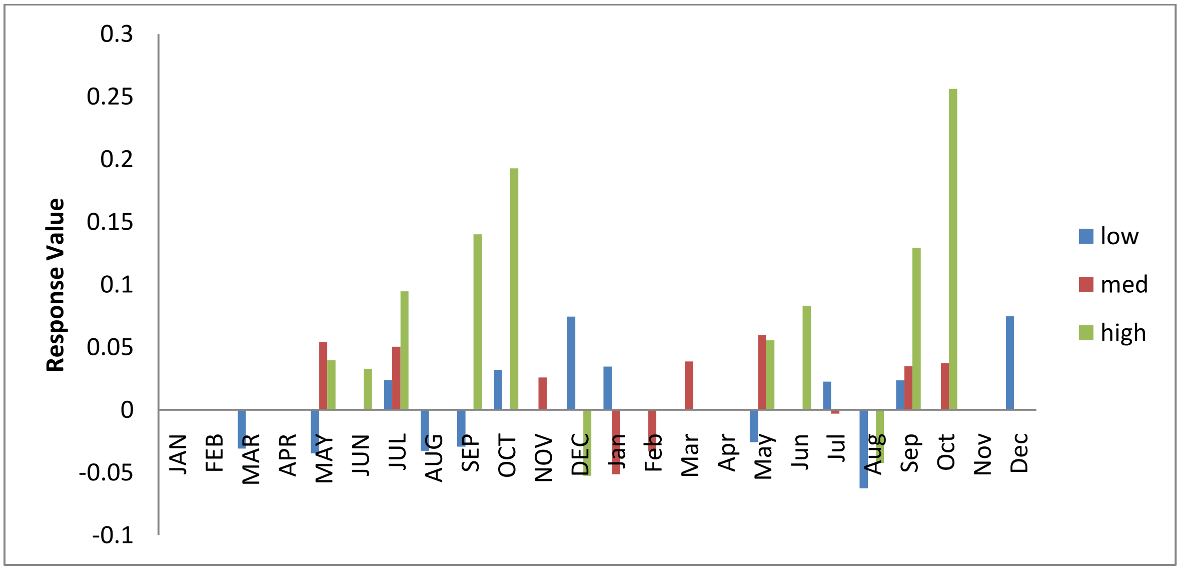


Table 3.4: Load coefficients of the five significant variables for LD functions 1 and 2 of an analysis of 21 aspen stands in northern Minnesota.

Standardized Canonical Discriminant Function Coefficients

	Function	
	1	2
Variance Explained	72%	28%
QMD	1.892	-5.468
Relative Aspen Density	4.777	-1.336
Relative Oak Dominance	1.379	.493
Diversity	13.592	4.213
Species Richness	-5.629	-.787

Figure 3.4: Ordination of 21 aspen stands in northern Minnesota using linear discriminant analysis. Numbers to the right of each point indicate the level of stand vigor (0 = low, 1 = medium, 2 = high).

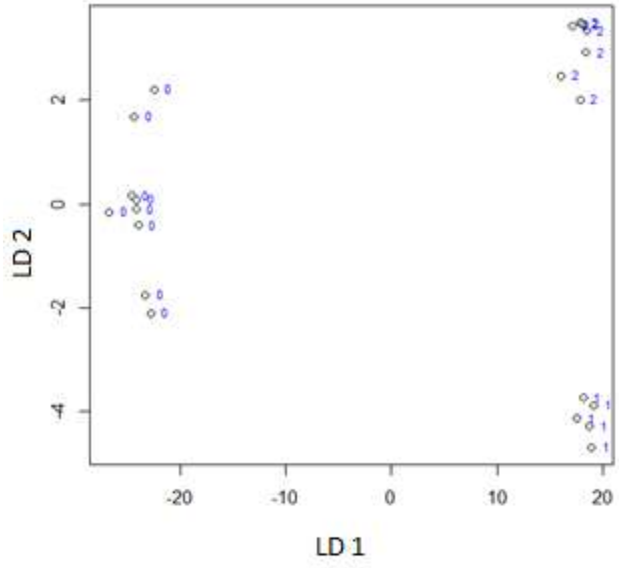


Table 3.5: The distribution of three vigor classes (low, medium, and high) according to geographical location. PBEF = Pike Bay Experimental Forest, MEF = Marcell Experimental Forest, and NEM = northeast Minnesota.

	Vigor		
Location	Low	Medium	High
PBEF	0	3	4
MEF	1	2	2
NEM	8	0	1

Figure 3.5: Average response of aspen stand BAI to monthly temperature over the current and previous year for each of three geographic locations in northern Minnesota. PBEF = Pike Bay Experimental Forest, MEF = Marcell Experimental Forest, and NEM = northeast Minnesota.

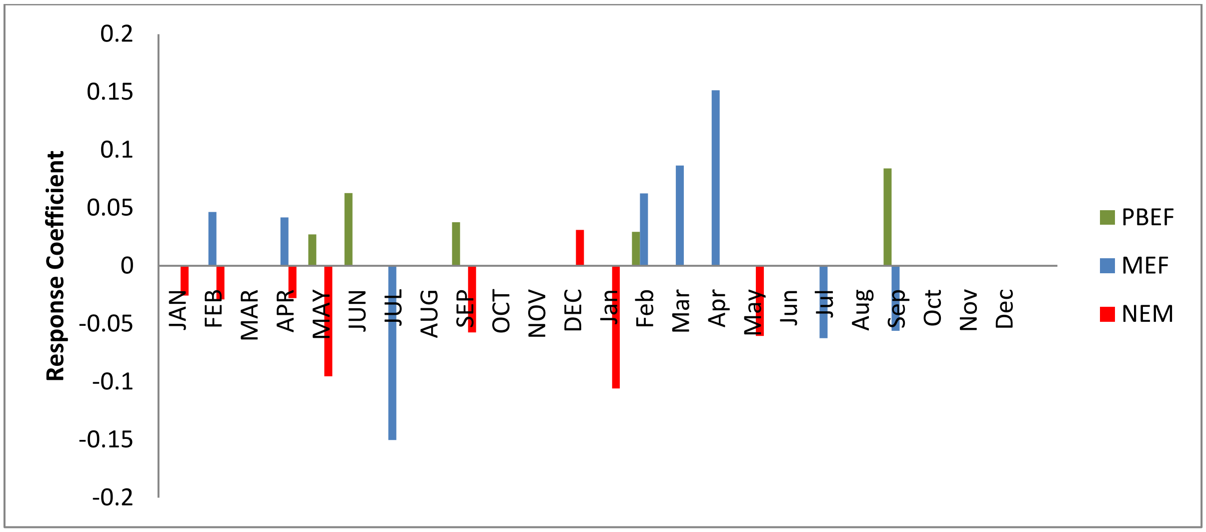


Figure 3.6: Average response of aspen stand BAI to monthly precipitation over the current and previous year for each of three geographic locations in northern Minnesota. PBEF = Pike Bay Experimental Forest, MEF = Marcell Experimental Forest, and NEM = northeast Minnesota.

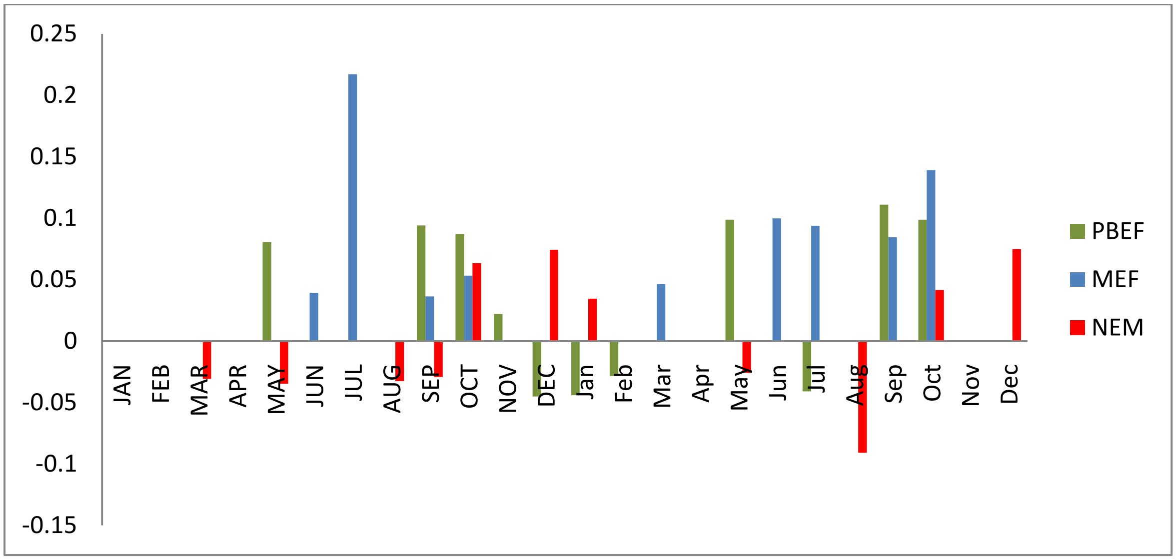


Table 3.6: Group averages of significant (α = 0.1) stand characteristics with corresponding F-statistics and p-values used to discriminate between stands that respond to temperature and precipitation variables and those that do not.

Trait	Responsive	Unresponsive	F	p
	Temperature			
Relative Maple Dominance	0.23	0.54	5.28	0.03
Stand BAI	26.87	55.78	3.23	0.09
	Precipitation			
Stand Density	693.02	1023.9	3.82	0.07

APPENDIX V

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ABSTRACT

DIOECIOUS PLANTS: EVOLUTION AND SEX RATIO AND ASEPEN DECLINE

by

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Advisor: Dr. D Carl Freeman

Major: Biology

Degree: Doctor of Philosophy

Although dioecious species are rare in nature, the sexual system appears in a diverse number of families and across all life forms and global locations. We looked at the population dynamics of natural populations to determine why many dioecious populations display biased sex ratios. We also looked at the role incestuous matings, seed and pollen dispersal patterns, and compensation play in the evolution of this sexual system using two theoretical models we developed. Finally, we studied aspen populations in the Midwest to determine how climate variables affect growth and decline. We found many dioecious species display male biased ratios and that life form and dispersal agents are good indicators. Our theoretical models imply that incest and dispersal specialization of unisexual individuals facilitate invasion, but through the interaction of specialization, incest, and compensation, unisexual invasion occurs much more consistently and under less stringent conditions then previously modeled. Finally, we found that declining aspen stands were much less responsive to climate variables than healthy stands and that a number of stand characteristics could be used to discriminate between responsive and non-responsive stands.

AUTOBIOGRAPHICAL STATEMENT

I arrived at Wayne State in the fall of 2002 as a Mathematics major and a member of the softball team. Exactly 10 years later I am leaving with a BS and MA in Math, a PhD in Biology, and a lot of great memories. Detroit - and Wayne State in particular - has been a wonderful place to spend my twenties.