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THE GENETICS AND EVOLUTION OF SUBDIOECY IN ASTILBE BITERNATA

by

Matthew Scott Olson

Department of Botany
Duke University

Date: November 24, 1997

Approved:

Janis Antonovics, Advisor

Dissertation submitted in partial fulfillment of
the requirements of the degree of Doctor of Philosophy
in the Department of Botany
in the Graduate School of Duke University

1997
ABSTRACT

(Botany)

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William F. Morris

Epiphic P. Lyons

Paul Mann

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Abstract

The Genetics and Evolution of Subdioecy in *Astillbe biternata*

All populations of *Astillbe biternata* have different individuals which express male, female, and hermaphrodite phenotypes. This breeding system has been termed subdioecy by previous researchers. Because all other *Astillbe* species are hermaphroditic and *A. biternata* is a tetraploid which presumably arose from the hybridization of two unknown diploid *Astillbe* species, it is assumed that the ancestors of *A. biternata* were hermaphroditic. Both the pathway along which the breeding system is evolving and the trajectory of evolution in *Astillbe biternata* are assessed.

The distributions of the quantitative genders of individuals in populations at Coweeta Hydrological Laboratory and patterns of gender transitions of individuals at Coweeta and in the Duke University greenhouses were used to deduce that subdioecy in *A. biternata* is evolving along the gynodioecious pathway between hermaphroditism and dioecy. Furthermore, clonal repeatability studies suggest that there is genetic variation for the propensity to set fruit in hermaphrodites and crossing studies show that there is genetic variation for the propensity to produce a full complement of stamens in the inflorescence. These observations of genetic components to gender expression as well as field observations suggesting a negative genetic correlation between stamen production and fruit production support assumptions of theoretical models of the evolution along the gynodioecious pathway.

Fruit set of females was found to be over four times as high as fruit set in
hermaphrodites. Moreover, outcross siring success of males was slightly higher than for hermaphrodites; however, when the evolutionary trajectory was placed in the context of theoretical models, the siring success of males was not sufficiently high compared to hermaphrodites to predict the maintenance of males in populations. Therefore, the relative reproductive outputs of males, females, and hermaphrodites suggests that future evolution of the breeding system will be towards gynodioecy. However, this conclusion must be viewed with caution because the inheritance of factors determining male sterility is complex. Future studies, some of which are already in progress, will be devoted to better understanding the genetics of sex-determination in *A. biternata*.

Genetic studies of the inheritance of allozyme markers suggest that *A. biternata* is an allotetraploid, formed from a hybridization event between two unknown diploid species of *Astilbe*. It is suggested that this hybridization event may have initiated the shift in breeding systems from an ancestral hermaphroditic condition to the present subdioecious breeding system.
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CHAPTER 1

Historical perspective and overview of research
Charles Darwin in “The Different Forms of Flowers on Plants of the Same Species” (1877) was among the first to note that in plants there was a continuum of breeding systems between hermaphroditism and dioecy. Of particular interest is his characterization of the breeding system of *Euonymus europaeus*, which consists of females, hermaphrodites, and males. After observing the sex-expression of several plants over several seasons, Darwin found that the same individual could express a hermaphrodite phenotype in one year and a male phenotype in a different year, but females produced fruit in every year. He termed this breeding system variously trioecious, subdioecious, or polygamous. And remarks “this case ... [shows] how gradually an hermaphroditic plant may be converted into a dioecious one (p.292).”

One hundred and twenty years after Darwin’s observations, the selective and genetic complexities of how and why species evolve along the continuum from hermaphroditism to dioecy still remain a mystery. As with many subjects in evolutionary biology, the theoretical developments in this area are far ahead of the empirical understanding of the process. An Evolutionary Stable Strategy (ESS) model by Charnov, Maynard Smith, and Bull (1976) was the first to rigorously discuss the fitness tradeoffs and total fitnesses of hermaphrodites relative to unisexuals (males and females) which were theoretically necessary for the evolutionary transition from hermaphroditism to dioecy to occur. This model was later expanded by Charlesworth and Charlesworth (1981) to include selfing in hermaphrodites. Both models assumed that there was a genetic component for directing the relative allocation of resources to pollen and ovules
and that there were “fitness tradeoffs” derived from a specific allocation strategy. These models suggested that dioecy should evolve from hermaphroditism when the fitnesses of each of the unisexuals exceed the fitness of hermaphrodites. However in regard to subdioecious species, these models could explain the simultaneous presence of males, females and hermaphrodites only in the “artificial and unlikely case [of equal total fitnesses for all three genders] (Charnov et al. 1976).”

Charlesworth and Charlesworth (1978) expanded the theoretical understanding of the evolution of subdioecy by explicitly incorporating genetics of the male and female sterility factors and allowing for self-fertilization in hermaphrodites. Their model was a breakthrough in being the first evolutionary model to explicitly incorporate genetic assumptions regarding proto-sex-determination. It assumed that there were two nuclear genes at different loci which affected male and female sterility, respectively. If the total fitness through ovules of the male sterile was higher than the fitness through ovules of the hermaphrodites (conditions similar to those outlined by Charnov et al. 1976), the male sterility factor could invade, resulting in a gyn dioecious breeding system (hermaphrodites and females). Subsequently, a female-sterility factor could invade if it conferred increased siring success. By assuming male and female sterility factors were determined by different loci on the nuclear genome, their model predicted the segregation of hermaphroditic and neuter progeny from crosses between males and females as a result of recombination between sex-determining loci. Without complete linkage disequilibrium between male and female sterility loci, some hermaphrodites (male and female fertile) and some neuters (male and female sterile) would be continually
reintroduced into the population. Subdioecy would be maintained when unisexuals are favored over hermaphrodites by having higher total fitnsses than hermaphrodites and when sex-determining loci were not completely linked. Moreover, the breeding system is predicted to remain subdioecious until either complete linkage disequilibrium between male and female sterility factors evolve (resulting in complete dioecy) or conditions change so that males, females, or both no longer had higher fitnesses than hermaphrodites, resulting in the loss of at least one unisexual gender.

Although the Charlesworth and Charlesworth (1978) model was an innovation, their assumption that male-sterility was inherited by factors in the nuclear genome was challenged by empirical evidence from gynodioecious species. Empirical studies of the inheritance of male-sterility in gynodioecious species were finding that in most cases male-sterility was determined by a combination of cytoplasmic and nuclear factors (Kheyr-Pour 1980, Van Damme 1983, Couvet et al. 1986, Belhassen et al. 1991). In particular, it was argued that cytoplasmic gynodioecy may not be able to evolve to full dioecy (Ross 1978).

Fifteen years later, Maurice et al. (1993) developed the first model which showed that evolution from cytoplasmic gynodioecy to complete dioecy was theoretically possible (to be fair Schultz 1994 may have come to the same conclusion independently, and at the same time). Moreover, cytoplasmic inheritance of male-sterility was shown to facilitate the maintenance of males in populations relative to nuclear inheritance (Maurice et al. 1994). Interestingly, in almost all cases (see Maurice 1992 for exception), cytoplasmic determination of male sterility was shown to be lost before complete dioecy evolved.
because all but one male-sterility cytoplasm remained when dioecy evolved. When more than one cytoplasmic male-sterility factor remained in the population, subdioecy (i.e. males, females, and hermaphrodites) was likely to be the final equilibrium breeding system (Maurice et al. 1993, 1994, Schultz 1994, but see Maurice 1992).

The inclusion of cytoplasmic male sterility in the models by Maurice et al. (1993) and Schultz (1994) necessitated inclusion of many additional parameters (e.g. the type of male-sterility cytoplasm, the cost of restoration of a male-sterility cytoplasm, and the cost of restorers when the male sterility cytoplasm is not present). Hence, the complexity of the models increased dramatically. Although theoretically tenable, these models are difficult to test empirically because analytical solutions which describe the relative contributions of different parameters to the evolution of the breeding system are not available. Therefore, unless analytical models can be developed, case-specific numerical models will have to be used to place empirical results into the context of theoretical models.

**Empirical studies**

As was noted previously, empirical studies of the evolution of subdioecious breeding systems in plants have lagged behind theoretical developments in the field. To date, only a handful of subdioecious species have been studied in any detail. In subdioecious species, it is common to find that fruit production in females is much higher than that in hermaphrodites (Del Castillo 1986, Sakai and Weller 1991, Fleming et al. 1994, Wolfe and Shmida 1997). However, because only one study (Del Castillo 1986) has estimated both selfing rates and inbreeding depression in hermaphrodites, the relative
fitness of the seeds which are produced by females and hermaphrodites is often unknown. Similarly, comparisons of reproductive success via pollen in males and hermaphrodites have not been adequate, usually involving comparisons of relative pollen production of different individuals. In general, the best estimates of siring success are direct measurements made by following genetic markers from sire to seed. Although measures of pollen production are much easier than direct measurements of siring success, they can be misleading (Meagher 1991, Stanton et al. 1992, Snow and Lewis 1993). Comparisons of relative pollen output have produced varying results. Two have found that pollen production is higher in males than in hermaphrodites (Fleming et al. 1994, Wolfe and Shmida 1997) and one has shown that there is no difference in pollen production (Sakai and Weller 1991). Prior to the data presented in Chapter 3 of this dissertation, there have been no comparisons of relative siring success of males and hermaphrodites in subdioecious species using genetic markers.

The data on the presence of genetic variation for genetic sex-expression is similarly lacking among subdioecious species. Aside from one study which found genetic variation for fruit production in hermaphrodites (Delph and Lloyd 1991), there are no studies documenting genetic variation for gender expression in subdioecious species.

**OVERVIEW OF THESIS**

In my research I set out to develop an understanding of the factors important for the evolution of the breeding system in *Aristolochia biformis*, a subdioecious plant species. I therefore gathered data on genetic and environmental influences on sex-expression in hermaphrodites, on the reproductive output of males, females, and hermaphrodites
(including direct measures of siring success), and estimates of selfing rates and inbreeding depression. These data were then used to assess the evolutionary trajectory of *A. biternata* and broaden our understanding of the empirical basis for evolution of gender in subdioecious species.

Early in my tenure at Duke, I was struck by the wide variety of gender expression displayed by *Aristolochia biternata* in populations at Coweeta Hydrological Laboratory. Much like *Euonymus europaeus*, different individuals of *A. biternata* displayed male, female, and hermaphroditic genders. In Chapter 2, I describe the extent of the variation in gender expression of *A. biternata* on many scales, including results based on studies of population sex ratios, variation in phenotypic gender within populations, variation in gender expression within individuals, and whether genetic factors affect reproductive output through male and female function in hermaphrodites.

In Chapter 3, I present the results from studies which compare the reproductive output of males, females, and hermaphrodites. The estimates of reproductive output are then compared to other subdioecious species and to the predictions from theoretical models of the evolution of subdioecy.

In Chapter 4, I present the results of a study of the genetics of inheritance of allozyme markers in *A. biternata*. The results support an earlier hypothesis by Hamel (1953) that *A. biternata* is an allotetraploid, derived from the hybridization of two unknown diploid ancestors. In addition, during months and months of allozyme gel development and analysis of banding patterns, a bit of lagniappe (Cajun word for "a little something extra") fell into my lap and refreshed my intellectual curiosity. The result was
a Bayesian method for discriminating among multiple hypotheses for inheritance of traits or alleles using categorical data. Until my work, it was common, in cases where multiple hypotheses were being compared, to do independent goodness-of-fit tests, comparing the distribution of the data against each hypothesis separately. Philosophically, this approach is not as satisfying as a test that determines the probability that one of the hypotheses is true given the data. In Chapter 4, I therefore present a Bayesian approach for testing among multiple hypotheses and apply it to the problem of testing among several hypotheses concerning allozyme banding patterns in A. biternata.

In the final Chapter, I summarize the results of the studies presented in this thesis and provide direction for future theoretical and empirical studies of the evolution of subdioecy and dioecy in plants.
CHAPTER 2

Patterns of sex expression in subdioecious Astilbe bibernata
INTRODUCTION

The evolutionary pathway along which a breeding system is evolving is an essential component of understanding the evolutionary processes which result in the evolution of dioecy from hermaphroditic ancestors. In plants, theoretical models suggest dioecy can evolve from hermaphroditism along three pathways (Lloyd 1980a, Ross 1982). First, dioecy may evolve from hermaphroditism along a gynodioecious pathway whereby complete male sterility mutations appear and reach equilibrium in a population (gynodioecious intermediate with females-♀ and hermaphrodites-[♂♀]) before female-sterility mutations are introduced (Charlesworth and Charlesworth 1978, Ross 1982). Alternatively, dioecy may evolve from hermaphroditism along an androdioecious pathway whereby complete female sterility mutations persist (androdioecious intermediate with males-♂ and hermaphrodites-[♂♀]) before male sterility mutations are introduced (Charlesworth and Charlesworth 1978, Ross 1982). Finally, both partial male sterility and partial female sterility modifiers could enter the hermaphroditic population (hereafter referred to the hermaphroditic intermediate model) with the male-biased gender evolving increased maleness and the female-biased gender evolving increased femaleness (Ross 1982). The evolutionary pathway through gynodioecy has been argued to be the route to dioecy in several species (Delph 1990a, Delph and Lloyd 1991, Weller and Sakai 1990, Wolfe and Shmida 1997, Desfeux et al. 1996) and, therefore, is thought to be the most common of the three pathways by which dioecy evolves from hermaphroditism (Lloyd 1980a, Charlesworth and Charlesworth 1978).

The hermaphroditic intermediate model differs from the other two models in two
ways. First, the male and female sterility factors do not completely abolish the production of pollen and ovules, respectively, when introduced in the hermaphroditic intermediate model; whereas, in the gynodioecious and androdioecious intermediate models the male and female sterility factors completely halt stamen and ovule production, respectively. Second, both proto-males and proto-females of the hermaphroditic intermediate can self-fertilize, whereas the females in the gynodioecious intermediate and the males in the androdioecious intermediate cannot self-fertilize.

Since dioecy and subdioecy (breeding system consisting of $\sigma^+$'s, $\varphi^-$'s, and $(\sigma \varphi^-)$'s) are common to all three pathways, the presence of dioecy or subdioecy provides no insight into the evolutionary pathway between hermaphroditism and dioecy. Two types of information have been used to infer the evolutionary pathway of dioecy and subdioecy: 1) the taxonomic associations between a species with a dioecious or subdioecious breeding systems and a species with an intermediate breeding system and 2) gender patterns associated with populations and individuals. These will be discussed in turn.

In some cases dioecy and gynodioecy co-occur within genera. This information has been used to postulate that dioecy evolved along the gynodioecious pathway in *Hebe* (Delph 1990a), *Schiedea* (Weller and Sakai 1990), and *Silene* (Desfeux et al. 1996). However, if no species with gynodioecy or androdioecy exit within a genus (e.g. dioecy or subdioecy may be unique to one species within a genus), taxonomic associations do not provide evidence to support one pathway over another.

For subdioecious species it is possible to infer the evolutionary pathway from information on the patterns of quantitative gender variation within populations (Lloyd
Quantitative estimates of gender take into account the relative contributions of pollen and ovules to the gamete pool of the population, whereas morphological gender does not account for the relative fitness gained via pollen and ovules. For this reason, quantitative gender describes the relative capabilities of plants as pollen and ovule parents more accurately than morphological descriptions of gender (Lloyd 1980a). For instance, in gynodioecious species, hermaphrodites contribute all of the pollen for fertilization of ovules and only a portion of the ovules available for fertilization. Because each seed has one pollen parent and one ovule parent, hermaphrodites in gynodioecious populations contribute more gametes to the next generation via pollen than ovules. Patterns of the distributions of quantitative genders of individuals in populations may be useful for predicting the evolutionary pathway along which subdioecious species are evolving (Lloyd 1980a). Once dioecy has evolved, the gender distribution patterns are the same (only complete males and females) regardless of the pathway to dioecy. Predictions for patterns of gender distributions associated with each pathway for the evolution of dioecy have been developed and are outlined below.

With subdioecious species it is also possible to infer the evolutionary pathway by observing gender transitions of individuals. Gender transitions are changes in the expression of gender of an individual and can result in transitions from male to hermaphrodite or female, female to male or hermaphrodite, or hermaphrodite to male or female. This type of plasticity of gender expression is common among species with breeding systems along the evolutionary continuum between hermaphroditism and dioecy and should not be confused with diphasy or sex-choice (Lloyd and Bawa 1984) which
occurs in species such as *Arisaema triphyllum* (Bierzychudek 1984, St. Amour 1997) and *Acer pennsylvanicum* (Hibbs and Fischer 1979). Diphasic species change their gender in response to increased size or age and all individuals are capable of switching sex. In contrast, for species in evolutionary transition between hermaphroditism and dioecy, adjustments in gender result from phenotypic plasticity and gender adjustments may not occur in all genders. For instance, females may always remain female, while their males may switch to hermaphrodites and vice-versa. Predictions for patterns of gender transitions associated with each pathway for the evolution of dioecy are outlined below.

Whether a species is on one pathway or another does not imply which direction the subdioecious species is evolving. For instance, a subdioecious plant on the gynodioecious pathway may have first evolved dioecy, and subsequently, hermaphrodites may have reinvaded the population when male mutants regained the ability to produce some seeds. A similar scenario has been proposed for androdioecious *Datisca glomerata* whereby androdioecy was derived from dioecy by females regaining the ability to produce pollen (Rieseberg *et al.* 1992).

**Predictions for Gender Distributions and Gender Transitions—**

**GYNODIOECIOUS PATHWAY** - When intermediates are gynodioecious, theoretical models assume that two types of genetic mutations must arise: one mutation causes the loss of reproduction through pollen and a second mutation causes loss of reproduction through ovules (Westergaard 1953, Charlesworth and Charlesworth 1978). First, a male-sterility factor enters a population and persists creating a breeding system with females and hermaphrodites (gynodioecy; Charlesworth and Charlesworth 1978). This male-sterility

The expected distribution of the quantitative genders of individuals in subdioecious populations evolving along the gynodioecious pathway would consist of two classes of genders. First, a class of invariant females would be present. The second class would consist of hermaphrodites which vary in their relative ovule production (Lloyd 1980a). These hermaphrodites would have a range of quantitative genders. Hermaphrodites which produce many seeds would have more female-like quantitative genders while which produced few or no seeds would have more male-like quantitative genders. Therefore, in the population there a range of quantitative genders would be expected (Lloyd 1980a).

Individuals in species with subdioecious breeding systems evolving along the gynodioecious pathway would exhibit gender adjustments between male and hermaphrodite genders and not between female and male or female and hermaphrodite genders (Lloyd 1980a). This pattern in gender adjustment theoretically results from
greater plasticity in sex-expression within hermaphrodites than females (Sutherland and Delph 1984, Delph 1990b).

**Androdioecious Pathway** - When dioecy evolves through androdioecious intermediates, female sterility mutations enter the hermaphroditic population (resulting in an androdioecious breeding system) prior to the entrance of male-sterility mutations. For subdioecious species evolving along the androdioecious pathway, males would be invariable in their gender expression and hermaphrodites would vary in their stamen production. Thus, the distribution of the quantitative genders of individuals in subdioecious populations along the androdioecious pathway would consist of a class invariant males and a class of hermaphrodites which vary in their relative stamen production with a corresponding range of phenotypic genders (Lloyd 1980a). Patterns of gender transitions for individuals in species with subdioecious breeding systems evolving along the androdioecious pathway would exhibit changes primarily between female and hermaphrodite genders and not between male and female or male and hermaphrodite genders (Lloyd 1980a).

**Hermaphrodite Intermediate Pathway** - When subdioecious species have evolved from hermaphrodites which are modified by partial male and female sterility factors, proto-males (individuals with partial female sterility factors) would express genders ranging from males which produce no seeds to hermaphrodites which produce a few seeds (Ross 1982). Likewise proto-females (individuals with partial male sterility factors) would express genders ranging from females which produce no pollen to hermaphrodites which produce reduced amounts of pollen (compared to males; Ross
1982). Gender transitions in subdioecious species along the hermaphrodite intermediate pathway would be expected to occur between females and hermaphrodites, and between males and hermaphrodites, but not between males and females (Ross 1982).

**SUBDIOECY IN ASTILBE BITERNATA** - In this chapter I characterize subdioecy in *Astilbe biternata*, a herbaceous plant in the Saxifragaceae in which males, females, and hermaphrodites can be found in every large population. To give a sense of the variation in the breeding system in *A. biternata*, I first describe patterns of sex ratios in populations throughout the range of the species and then describe patterns in sex ratios over time within two populations. I proceed to examine the distribution of gender within populations as well as patterns of gender transitions for individuals. These patterns are used to argue that *A. biternata* lies along the continuum from gynodioecy to dioecy.

**STUDY SPECIES**

*Astilbe biternata* (Vent.) Britton is a herbaceous perennial in the Saxifragaceae which is endemic to the southern Appalachian Mountains, ranging from northern Georgia to southern West Virginia. It typically grows in the shaded understory of rich mesic cove forests and along nearby roadsides. The flowers of *A. biternata* are small and hundreds to thousands of them are borne in a large determinate inflorescence which varies between 10 and 80 cm in length. The inflorescences are arranged in loosely branched panicles which bear flowers on primary, secondary, and tertiary branches (Mellichamp 1976).

Along roadsides, individuals can produce large clumps of flowering stems from a single corm. However, in understory populations individuals rarely produce more than
one inflorescence per year. *Aristolbe biternata* flowers in late June and early July and sets fruit by late July. After the first freeze in the fall, above ground stems die back. In the spring, new shoots arise from the overwintering corm.

The genus *Aristolbe* contains approximately 25 species most of which are found in southeast Asia (Mellichamp 1976). *Aristolbe biternata* is the only species in the genus which is native to North America. All *Aristolbe* species except *A. biternata* have bisexual flowers whereas *A. biternata* has both unisexual and bisexual flowers. Evidence on inheritance patterns of allozyme alleles (see Chapter 4) and cytology (Hamel 1953) suggest that *A. biternata* is an allotetraploid derived from the hybridization of two unknown diploid *Aristolbe* species.

**Gender expression**: In *Aristolbe biternata* populations individuals display one of three sex expression phenotypes in any specific year: male, female, and hermaphrodite. Females produce only ovules (Figure 2.1A). Males are morphologically hermaphroditic. They produce reduced gynoecia (compared to females) and stamens with viable pollen. Males do not produce seeds (Figure 2.1B) and it is not known whether the males produce ovaries. Hermaphrodites also produce gynoecia and stamens with viable pollen and at anthesis hermaphrodites are morphologically indistinguishable from males (Figure 2.1C, D); however, on hermaphrodites, ovaries develop into viable seeds in at least a portion of the flowers in the inflorescence. Because both males and hermaphrodites produce viable pollen but there is no apparent morphological distinction between the two types of flowers at anthesis and before seeds have developed, I refer to males and hermaphrodites collectively as "male-fertiles".
METHODS

FIELD SEX RATIOS - During 1994 and 1995 several populations throughout the range of *A. biternata* were visited and censused for population sex ratios, proportion fruit set of hermaphrodites and females, and the length of inflorescences of all flowering individuals. Fifteen populations were censused in 1994 and 20 were censused in 1995. Of these populations, thirteen were censused in both years. In each population, at least 100 individuals were censused along several transects spanning the population. Since most populations were adjacent to roadsides, the starting positions for transects were randomly located along the roadside and transects ran perpendicular to the road. If populations contained fewer than 100 flowering individuals, all individuals were censused. If populations were visited when both senesced stamens and developing fruit could be observed simultaneously, the population was visited only once during the growing season. If fruit were not developing during the first census, the population was revisited later in the growing season to determine whether male-fertile individuals had set seed.

Longer-term sampling of marked individuals at Coweeta Hydrological Laboratory was undertaken from 1994 through 1997. In two populations, Shope Fork and Ball Creek, over 650 *A. biternata* plants were censused and monitored for flowering and sex expression each year from 1994 to 1997. The Shope Fork population was located at the junction of Shope Fork Road (751) and Forest Service road 83-D. The Ball Creek population was located in the drainage basin of Reynolds Branch of Ball Creek between Reynolds gap and the intersection of Reynolds Branch and Ball Creek Road.
At Coweeta, when individuals flowered they were marked with flags so their gender expression could be followed in the future. Each year, both the marked plants and all additional flowering individuals were censused and marked with flags. For the first three years of the census individuals were scored for the presence or absence of stamens, their percent fruit set, and the length of their inflorescence. In 1997, percent stamen production and percent fruit set (see below) were estimated.

Hypotheses regarding variation in sex ratios among populations and among years were tested using PROC FREQ in the SAS for Windows statistical package (SAS Institute 1993). Likelihood ratio chi-square statistics were reported for one-way comparisons. For the two-way comparison of the interaction of the effects of year and population on sex ratio, the Cochran-Mantel-Haenszel (CMH) general association statistic testing the null hypothesis of no association in sex ratios between populations and years was reported. In general, CMH statistics have low power; therefore, caution should be exercised when concluding that there is no underlying association when the null hypothesis is not rejected (SAS Institute 1993).

If sex expression is determined genetically, when comparing the sex ratios across several populations, the proportion of females is expected to be inversely related to the number of male-fertile individuals which produce seeds (i.e. \( \frac{\text{hermaphrodites}}{\text{males} + \text{hermaphrodites}} \), Lloyd 1976). This hypothesis was tested for A. biennata populations in 1994 and 1995 by testing whether the slope of the linear regression of the frequency of females on the frequency of male-fertiles which produce seeds was negative and significantly different from zero (SAS Institute 1993).
QUANTITATIVE ESTIMATES OF GENDER - ESTIMATION OF FRUIT SET AND STAMEN

PRODUCTION ON INDIVIDUAL PLANTS - Because A. biternata inflorescences contain thousands of flowers, determining the exact fruit set and stamen production for hundreds of individuals in situ was infeasible. Therefore, the following methods were developed to obtain quick estimates of the percent fruit set and percent stamen production for each individual.

In 1995, fruit set of hermaphrodites and females was compared in 11 populations covering the range of A. biternata. Each inflorescence was assigned a percent fruit set to the nearest 10% based on a visual assessment of the proportion of the number of flowers which developed fruit. Percent fruit set was assigned to one of the 13 following categories: 0, 2, 10, 20, 30, 40, 50, 60, 70, 80, 90, 98, or 100%. At Coweeta, inflorescences with between 10 and 90% fruit set were revisited to check the accuracy of initial assessments. In other populations only a subset of the individuals (approximately 10 individuals) were revisited to check the accuracy of initial estimates.

At the Ball Creek and Shope Fork populations, the relative stamen production was also assessed for all male-fertile individuals. Male-fertile A. biternata flowers produce at most 10 stamens each. In some male-fertile individuals stamen production per flower varies from 0 to 10 stamens. An appraisal which was analogous to that for percent fruit set was developed for percent stamen production. Each inflorescence was assigned a percent stamen production to the nearest 10% based on a visual assessment of the proportion of the stamens produced on the entire inflorescence (relative to the maximum of 10 stamens per flower). Percent stamen production was assigned to one of the 13
following categories: 0, 2, 10, 20, 30, 40, 50, 60, 70, 80, 90, 98, or 100%. Inflorescences with between 10 and 90% stamen production were revisited to check the accuracy of initial assessments.

Estimation of relative flower numbers and fruit set - To calculate both the relative numbers of flowers and relative fruit set for males, females and hermaphrodites, the following traits were measured: flowering frequency by year, inflorescence length, the relationship between inflorescence length and the number of fruit per inflorescence, and the number of seeds per fruit from females and hermaphrodites. Flowering frequencies of males, females, and hermaphrodites were estimated from census records at Coweeta. For this analysis I called an individual a hermaphrodite if that individual produced both stamens and fruit in any of the four flowering seasons when data were collected. All plants which flowered in 1994 were analyzed for the number of times they flowered over the next 3 years at Shope Fork and the next two years at Ball Creek. The 1997 census at Ball Creek was not used because many flags had been removed by logging operations and many individuals could not be re-identified. A chi-square goodness of fit test (Steel and Torrie 1980) was used to test the null hypothesis that similar numbers of males, females, and hermaphrodites flowered more than once during the census period.

Quadratic curvilinear regressions were used to determine the relationships between the log₁₀ of the flower number and the inflorescence length. Regressions were made for males, females, and hermaphrodites using data from 33 male, 26 female, and 15 hermaphrodite inflorescences collected from plants in the US74 and Pearson Creek populations as well as plants grown in pots at Coweeta hydrological laboratory which
originated in the Ball Creek and US74 populations. An analysis of covariance was used to
test for homogeneity of quadratic curvilinear regression coefficients among populations
(US74, US80, and greenhouse), sexes, and sexes within populations (Zar 1984, Proc
GLM SAS version 6.1, SAS Institute 1993). Because the null hypothesis of homogeneity
of regression slopes was not rejected, all individuals were pooled to estimate regression
coefficients for the relationship between inflorescence length and the log_{10} of the flower
number (y = 2.10936+0.03957x-0.00022x^2; R^2=0.70; see Chapter 3, Figure 3.4).

The lengths of inflorescences produced by males, females, and hermaphrodites at
Coweeta were measured in 1995 and 1996. The null hypothesis of no difference in
inflorescence lengths between males, females, and hermaphrodites at Ball Creek and
Shope Fork was tested using an ANOVA with sex, year, and population as the main
effect treatments.

Seed set per fruit was estimated by counting seeds in locules (there are 2 locules
per fruit) from five hermaphrodites and four or five females each from the Ball Creek,
Shope Fork, US74, 226A and US80 populations. For each plant four to eight fruits
randomly selected from the inflorescence and seeds within one of the randomly selected
locule within the fruit was counted. Seed numbers per locule were compared with an
analysis of variance using PROC GLM (SAS Institute 1993). Seed numbers per locule
were doubled for estimates of seed numbers per fruit.

**Calculation of Quantitative Gender**: A quantitative estimate of gender was
computed for individuals at the Shope Fork and Ball Creek populations using methods
from Lloyd (1980) and Barrett (1992). Quantitative phenotypic gender (G_p) accounts for
the relative contributions of pollen and ovules from each gender class but does not account for the effects of inbreeding depression and selfing in hermaphrodites (Lloyd 1980b, Barrett 1992). To estimate $G_p$, it was assumed that all stamens on males and hermaphrodites produced equal amounts of pollen and that stamen production equated directly with fitness through pollen. $G_p$ was computed as:

$$G_p = \frac{d_i}{d_i + l_i E}$$

where $d_i$ is the proportion of fruit set for individual $i$ (see appraisal procedure above), $l_i$ is the proportion of stamen production for individual $i$ (see appraisal procedure above), and $E$ is an equivalence factor that scales the population-level contribution of pollen to equal the contribution through ovules (Lloyd 1980b). The equation to estimate $E$ is as follows:

$$E = \frac{\sum_{i=1}^{n} d_i}{\sum_{i=1}^{m} l_i}$$

where $n$ is the number of females in the population and $m$ is the number of male-fertiles (males+hermaphrodites) in the population. A gender value of 1.0 indicates that the individual reproduces solely via ovules, a gender value of 0.0 indicates reproduction occurs solely via pollen.

**Clonal repeatability** - Twenty genotypes, eight females and twelve male-fertiles (males or hermaphrodites) were used in studies to assess gender transitions and
clonal repeatability in the greenhouse. Eighteen of these genotypes were dug up from the Ball Creek population at Coweeta, placed in pots, and transferred to the greenhouses at Duke University in 1994. Two of the male fertile genotypes (100 and 102) were grown from seed collected from the same plant in the Ball Creek population. Genotypes were cloned by dividing corms during the early spring while plants were still dormant. Clones were placed in separate pots and grown. Most did not flower until 1996. Unless otherwise noted, plants were grown outside in a lath house which provided some shade, and were fertilized during the growing season by alternating weekly application of 15-0-15 and 20-10-20 Peters Professional fertilizer. During the early spring in 1997 while plants were dormant, all soil was washed from corms. Corms were weighed to determine if sex was associated with corm mass and repotted.

Two experiments were conducted to assess different aspects of consistency in sex expression. The first was designed to assess the effects of environmental variation on sex expression and to determine what types of gender transitions, if any, occur in Astilbe bibernata. Paired clones from 7 male-fertile and 5 female genotypes were randomly assigned to either a relatively high light/high nutrient environment (lath house/ fertilized once a week) or a low light/low nutrient environment (lath house + 80% shade cloth/ fertilizer once a month) for the entire growing season. At the time of flowering, inflorescences were inspected and stamens were noted as present or absent. Percent fruit set was estimated four to six weeks after fruits had developed (see above for methods of estimation). A Fisher’s exact test (SAS PROC FREQ, SAS Institute 1993) was used to test the null hypothesis of no effect of the environmental treatments on gender expression.
The second experiment was designed to determine the clonal repeatability in the propensity of male-fertile genotypes to set fruit. During the 1997 growing season, between four and eight clones from each of five male-fertile genotypes were grown in the same environment (lath house / weekly fertilization). To increase replication of two genotypes for the experiment, two large corms (one from genotype 102 yielded 3 extra clones and the other from genotype 100 yielded 2 extra clones) were divided during the early spring of 1997. The rest of the clones had been divided prior to the winter of 1996 and had subsequently grown in separate pots. At anthesis percent stamen production was estimated (see above). Percent fruit set was estimated several weeks after fruit had matured (see above). Because females had generally high fruit set (>95%) in the lath house, it was assumed that the pollinator fauna preformed adequate pollination for seed set on hermaphrodites. A Fisher’s exact test (SAS PROC FREQ, SAS Institute 1993) was used to test the null hypothesis that genotype had no effect on the propensity to produce fruit.

CROSSING STUDIES - To gain insight into the genetics of gender determination, in 1995 experimental crosses of males and females were performed in the greenhouse and in the field at Ball Creek, Shope Fork and the US74 populations. In all cases, peduncles of female inflorescences were bagged with cellulose tubing prior to the time when stigmas were receptive. Bags were removed only to pollinate the flowers. Whole flowers were removed from pollen donors and anthers were brushed several times across each receptive stigma to ensure pollination. To test whether foreign pollen could have entered when bags were removed, one bag was removed from the inflorescence on each female,
the flowers were left unpollinated (pollinators were kept away), and the bag was replaced approximately 5 minutes later. No flowers on these inflorescences developed mature seeds.

Seeds were allowed to mature on the inflorescence and planted in seedling mix in November 1995. During the spring of 1996 all individuals were transferred to 6" diameter pots and placed in the greenhouses at Duke University where they were fertilized weekly. Plants did not flower during their first growing season. During their second growing season all plants were placed in the lath house and fifty percent of the 550 plants from the crosses flowered. These plants were scored for both fruit set and percent stamen production.

RESULTS

FIELD SEX RATIOS THROUGHOUT THE RANGE OF A. BITERNATA - Overall sex ratios combining all populations were 0.51 : 0.40 : 0.09 (males♂:females♀:hermaphrodites♂♀) in 1994 (Table 2.1) and 0.51♂ : 0.39♀ : 0.10♂♀ in 1995 (Table 2.2). Combining populations, there was a bias toward higher frequencies of male-fertiles than females in both years (1994 $\chi^2$=76.6, df=1, $P<0.0001$; 1995 $\chi^2$=64.0, df=1, $P<0.0001$). In both years in the majority of the populations fewer than 50% of the flowering individuals were female (1994-14 of 15 populations contained fewer than 50% females, Table 2.1; 1995-16 of 20 populations contained fewer than 50% females, Table 2.2).

VARIATION BETWEEN 1994 AND 1995- The difference between overall sex ratios in
1994 and 1995 was not significant \( (\text{Likelihood ratio } \chi^2 = 0.83, \text{ df} = 2, P > 0.65) \). Likewise comparing populations which were sampled in both 1994 and 1995 there was no detectable difference in sex ratios among years (Cochran-Mantel-Haenszel (CMH) general association controlling for population differences, comparing M+H:F = 1.46, df=1, P > 0.20; comparing M:F:H - CMH general association= 3.6, df=2, P > 0.15).

**Variation among populations** - Sex ratio varied among populations (Tables 2.1 and 2.2; Figures 2.2 and 2.3; CMH controlling for differences between years, comparing M+H:F, General Association = 61.0, df=12, P < 0.001; comparing M:F:H, General Association = 125.9, df=24, P < 0.001). In 1994 proportions of males within populations varied from 0.35 to 0.66, proportions of females varied from 0.24 to 0.61 and proportions of hermaphrodites varied from 0 to 0.22 (Table 2.1). Eight of the fifteen populations had sex ratios which were significantly different from 1:1 (male-fertile:female; \( \chi^2 \) critical value adjusted for multiple comparisons \( \alpha = 0.05/15 = 0.0033 \); Table 2.1; Figure 2.2). In 1995 for populations with over 25 individuals, flowering proportions of males varied from 0.34 to 0.68, proportions of females varied from 0.17 to 0.53, and proportions of hermaphrodites varied from 0.01 to 0.28 (Table 2.2). Five of the twenty populations had sex ratios significantly different from 1:1 (male-fertile:female; \( \chi^2 \) critical value adjusted for multiple comparisons \( \alpha = 0.05/20 = 0.0025 \); Table 2.2; Figure 2.3).

The slope of the linear relationship between the proportion of male-fertile individuals which set seed and proportion of females in populations was negative, but not different from zero in 1994 and 1995 (1994 \( F_{1,10} = 1.51, P > 0.20 \); 1995, \( F_{1,14} = 0.98, P > 0.30 \); Figure 2.4).
FIELD SEX RATIOS AT BALL CREEK AND SHOPE FORK - OVERALL SEX RATIOS

ACROSS YEARS - Some of the plants at Ball Creek and Shope Fork flowered in more than one year. If a plant expressed a hermaphroditic phenotype in at least one year, it was considered a hermaphrodite for purposes of calculating the overall sex ratio across years at Ball Creek and Shope Fork. With this assumption, between 1994 and 1997, 259 males (39.3%), 322 females (48.9%), 75 hermaphrodites (11.4%), and 3 neuter (0.4%) individuals were identified at Ball Creek and 234♂ (49.7%), 214♀ (45.4%), and 23 [♂♀] (4.9%) individuals were identified at Shope Fork. Over all years, there was a slight trend toward male-fertile (males + hermaphrodites) biased sex ratios at Shope Fork ($\chi^2=3.93$, df=1, P<0.05) but not at Ball Creek ($\chi^2=0.22$, df=1, P > 0.50).

SEX RATIOS WITHIN YEARS - When sex ratios were computed within years, the sex of an individual was determined by its gender phenotype in the year in which it flowered irrespective of its phenotype in other years. When comparing the ratios of male-fertile phenotypes (males + hermaphrodites) to females within years, sex ratios were significantly different from 1:1 in only one year, in 1997 at Shope Fork (Table 2.3; $\chi^2$ critical value adjusted for multiple comparisons $\alpha=0.05/8=0.00625$). There was no statistically significant variation in sex ratio (male-fertile:female) over years at Ball Creek ($\chi^2=5.8$, df=3, P>0.10; Table 2.3) or Shope Fork ($\chi^2=3.7$, df=3, P>0.25; Table 2.3).

When hermaphrodites were considered a separate category from males, the sex ratio varied significantly among years (1994-1997) in the Ball Creek population (Table 2.3; $\chi^2=30.8$, df=6, P<0.001), but not at Shope Fork (Table 2.3; $\chi^2=8.9$, df=6, P>0.20).

At Ball Creek, hermaphrodite frequencies varied from a high of 19% in 1996 to a low of
5% in 1995 (Table 2.3). At Shope Fork hermaphrodite frequencies remained below 8% in every year (Table 2.3). Neuters, which were identified as individuals which produced inflorescences but neither pollen nor seeds even though pollen donors were in close proximity, were rarely seen. Three neuter individuals were observed at Ball Creek (Table 2.3), one in 1995 and two more in 1997.

FLOWERING FREQUENCIES- Flowering frequencies were low for all three genders (Table 2.4). Only 32% of plants that flowered in 1994 flowered at least once more over the subsequent 3 years at Shope Fork, and only 18% of plants which flowered in 1994 flowered again over the next 2 years at Ball Creek. For plants which flowered during the 1994 growing season, there was no statistical difference among males, females, and hermaphrodites in the frequency of plants that flowered at least once more in subsequent years (Table 2.4; Shope Fork, Fisher's exact test P=0.14; Ball Creek, Fisher's exact test P=0.75).

STAMEN PRODUCTION IN MALE-FERTILE INDIVIDUALS - In 1997, the majority of male-fertile individuals (100 of 123 at Ball Creek and 104 of 104 at Shope Fork) produced 10 stamens per flower on 100% of their flowers. Twenty-three male fertiles at Ball Creek had reduced stamen production. For these 23 individuals stamen production ranged from 2% to 80%. Overall, only 33% of male-fertile individuals with 100% stamen production produced fruit while 95% of male-fertile individuals with reduced stamen production set fruit.

There were 38 hermaphrodites at Ball Creek during 1997. Hermaphrodites with incomplete stamen production had an order of magnitude higher fruit set relative to
hermaphrodites with fully-formed stamens (means 54.2% vs. 4.2%, respectively; Kruskal-Wallis P<0.0001). Among hermaphrodites, there was a significant inverse relationship between percent stamen production and percent fruit set (Figure 2.5; Spearman’s Rho = -0.76, P<0.0001), suggesting a tradeoff in allocation to male and female reproductive output in hermaphrodites. At Shope Fork there were four hermaphrodites in 1997. All four had 100% stamen production and fruit set varied from 1 to 10%.

**Comparison of relative seed set of hermaphrodites and females** - There was no significant difference in the numbers of seeds per locule on females and hermaphrodites (Table 2.5; Females 11.2±0.7 (s.e.), Hermaphrodites 9.6±0.7 (s.e.)). In addition, inflorescence length did not differ significantly among sexes at either Shope Fork or Ball Creek (Table 2.6). There was a significant curvilinear relationship between the inflorescence length and the log of the flower number per inflorescence; however, this relationship did not differ among males, females, and hermaphrodites (Table 2.7; see Chapter 3 Figure 3.4).

**Quantitative gender estimates** - In 1997 all four hermaphrodites at Shope Fork had quantitative phenotypic genders ($G_p$) less than 0.2, suggesting that they obtained the majority of their reproductive success via pollen (Figure 2.6). In contrast, at Ball Creek 34% (13/38) of hermaphrodites were predicted to gain more than half their fitness through female function. $G_p$ estimates of hermaphrodites at Ball Creek ranged from 0.007 to 0.99 (Figure 2.6). Half of the hermaphrodites at Ball Creek had $G_p$ estimates of less than 0.1 indicating that if their reproductive success is proportional to the gametes they
produce, they gain over 90% of their fitness through male function.

**GENDER TRANSITIONS - NATURAL POPULATIONS** - A four year survey of 672 plants in the two populations at Coweeta revealed that females are invariant in their gender expression from year to year but males and hermaphrodites can change gender (Figure 2.7). In particular, at Shope Fork, 15 of the 69 (22%) males and hermaphrodites and, at Ball Creek, 22 of 77 (29%) males and hermaphrodites expressed male genders in some years and hermaphrodite genders in other years. Most male individuals (0% seed set) expressed a male gender in every year that they flowered (76% at Shope Fork and 57% at Ball Creek). Only a few hermaphroditic individuals expressed a hermaphroditic gender in every year that they flowered (2% at Shope fork and 14% at Ball Creek).

**EXPERIMENTAL OBSERVATIONS**: Results from greenhouse studies of clonal consistency in sex-expression in 1996 and 1997 reflected patterns seen in natural populations. In the greenhouse, all eleven female genotypes produced only pistillate flowers and 11 of the 13 genotypes which were male or hermaphroditic in the field in 1994 expressed both hermaphroditic and male genders on different clones of the same genotype at some time during the 1996 and 1997 growing seasons.

For male fertile genotypes, no significant relationship was found between the propensity to produce fruit and placement in high light/high nutrient or low/light low nutrient environments (Fishers Exact 2-tail; 1996 = 0.118; 1997 = 0.633). However, this conclusion must be viewed as tentative because the sample sizes in these experiments were small (there was often no replication of genotypes within environments) and the power of the experiments to detect differences among gender expression in the high light/
high nutrient and low light / low nutrient environments was low.

Clones of one genotype which was hermaphroditic in the field expressed both hermaphroditic and female genders in the greenhouse. This gender transition from hermaphrodite to female was the only transition that was not witnessed in the field. In 1996, all clones of this individual expressed very low stamen production (1%) and high fruit set (near 100%).

**Clonal repeatability:** Although the above studies show that males and hermaphrodites can adjust their genders from year to year, it indicates nothing about whether there is genetic variation present for the propensity of male-fertile individuals to set seed. In 1997 male-fertile genotypes differed in their propensity to produce fruit (Table 2.8, Fisher's Exact <0.001), suggesting that there is genetic variation for the propensity to produce seed among male-fertiles (Falconer 1989). Differences in the propensity to produce fruit could not be explained by differences in the masses of corms of different genotypes (see below). At least one clone of genotypes 102, 730, and 781 produced seeds, but no clones of genotypes 100 and 121 produced seeds. However, it is known that both genotypes 100 and 121 have the capacity to produce seeds because some clones of each genotype produced seeds in 1996.

**Relationship between size and gender expression** - In 1996, there was no association between gender expression and the weight of corms the winter after flowering (Mixed model ANOVA; F=0.0138, P>0.95; Figure 2.8). Likewise in 1997, there were no associations between the weight of male-fertile corms prior to flowering and their propensity to produce fruit (Mixed model ANOVA; F=0.803, P>0.35; Figure 2.9).
CROSSING STUDIES - Five out of eight of the crosses between males and females resulted in progeny ratios that were not significantly different from 1:1 (male-fertile:female; Table 2.9). Of the three crosses that produced sex ratios significantly different from 1:1, one was strongly male-hermaphrodite biased (123x103) and the other two (501x170, 501x787) were female biased (Table 2.9). No neuter phenotypes were observed. Because *A. biternata* is an allotetraploid and the sample size of crosses is small, it is imprudent to speculate about the possible genetic basis for sex-expression at this time.

One male (501) and one female (787) were included in two of the crosses presented here. Both progeny sets sired by 501 (Table 2.9; 501x170 and 501x787) had sex ratios that were biased toward females, but only one of the two sets of progeny in which 787 was the mother (Table 2.9; 501x787, but not 502x787) was biased toward females.

Reduced stamen production, a phenotype which was observed in the field, was significantly associated with crosses involving male 501 (Table 2.9; Fisher's Exact Test P= 0.0003). Of all other progeny, only one other male-fertile individual had reduced stamen production (Table 2.9).

DISCUSSION

GENDER TRANSITIONS - The patterns of gender transitions in *Aristolbe biternata* support the hypothesis that males are modified hermaphrodites which do not produce seeds. Gender modification appears to be the rule rather than the exception for male-
fertile individuals. In the field, male-fertile individuals produced stamens in every year and seeds in some years but not in others. In the greenhouse most male-fertile genotypes taken from the field were found to be capable of expressing hermaphroditic and male genders on different clones. Females, however, were strongly canalized in their sex-expression. In field populations, no female was every found to vary in its sex-expression. In the greenhouse no female genotype taken from the field ever produced stamens. This pattern of gender adjustment is consistent with a breeding system which is situated along the evolutionary continuum between gynodioecy and dioecy (Lloyd 1980a).

The patterns of gender transitions in *A. biternata* show similarities to what has been found in other subdioecious species along the gynodioecious pathway. For instance, in *Hebe subalpina* hermaphrodites are more variable in their annual fruit production than females (Delph and Lloyd 1991). Similarly, *A. biternata* male-fertiles often make transitions between making no seeds in one year to producing seeds in others while females produce seeds in every year in which they flower. In another subdioecious plant, *Schiedea globosa*, several individuals exhibited gender transitions between male and female genders in different flowering seasons (Sakai and Weller 1991). Gender transitions between male and female or between hermaphrodite and female are not predicted to occur in species evolving along the gynodioecious pathway (Lloyd 1980a). However, one *A. biternata* individual was also capable of expressing female and hermaphrodite genders in different years. These patterns suggest that although gender transitions from female to hermaphrodite and female to male are not common, they may be possible in subdioecious species along the gynodioecious pathway.
**Phenotypic Gender:** The patterns of standardized measures of phenotypic gender in *Aristolbe biternata* indicated that the majority of individuals expressed completely male or completely female phenotypes suggesting that the breeding system is close to dioecy. This is similar to patterns found in some populations of the subdioecious plant *Wurmbea dioica* (Barrett 1992). *Aristolbe biternata* appears to have a breeding system which is closer to dioecy than the breeding system of subdioecious *Gingidia montana* (Lloyd 1980a) and *Hebe subalpina* (Delph and Lloyd 1991); in both *G. montana* and *H. subalpina*, there are very few males which do not set fruit.

In dimorphic species where most male-fertiles set fruit, the standardized phenotypic gender of male-fertiles is biased toward reproduction though pollen (\(G_p < 0.5\)) because male-fertiles are the only sex class producing pollen while both male-fertiles and females are producing seed. Lloyd (1974) suggested that individuals with hermaphroditic sex-expression should be termed males, to stress that they gain the majority of their fitness via pollen. However, for *A. biternata* it may be misleading to call hermaphrodites males because one third of the hermaphrodites had gender estimates higher than 0.5 and are expected to gain most of their fitness through seeds. These female-biased gender estimates in hermaphrodites result from decreased stamen production. However, if longer-term estimates of standardized phenotypic gender for these individuals result in more male-biased gender estimates, Lloyd’s classification of hermaphrodites as males would also apply to *A. biternata*.

**Genetic and Environmental Influences on Sex-Expression - Gender**

Expression in *Aristolbe biternata* is governed, in part, by genetic characteristics. The
expression of female gender appears to be strongly canalized. Of the 11 female
genotypes transplanted from Ball Creek, none produced anthers when moved from field
to greenhouse environments. Genetic factors controlling femaleness appear to completely
disable stamen production.

On the other hand, both propensity to set fruit and stamen production were found
to be determined, in part, by genetic components in male-fertile individuals. In clonal
repeatability studies, certain male-fertile genotypes were more likely to produce fruit than
others, and in crossing studies, inheritance of less than 100% stamen production was
associated with a particular male parent.

Theoretical models of the evolution of male-fertile individuals along the
continuum between gynodioecy and dioecy (Charlesworth & Charlesworth 1978, Maurice
et al. 1993, 1994) assume that there are negative genetic correlations between
reproductive output through pollen and reproductive output through ovules in
hermaphrodites. The negative correlation between stamen and ovule production for
hermaphrodites at Ball Creek suggests such a tradeoff. Furthermore, findings of partial
genetic control of fruit set and stamen production in male-fertile individuals are not
inconsistent with the assumption of a negative genetic correlation. However, to rigorously
test this assumption it is necessary to determine relationships between parents and
offspring for a tradeoff in allocation to pollen and ovule production.

The presence of genetic variation for the propensity of male-fertiles to produce
fruit suggests that genetic modifiers affect set seed on male-fertile individuals
(Charlesworth and Charlesworth 1978, Delph and Lloyd 1991). When nuclear female-
sterility factors cause complete female-sterility, the crosses between males and females should produce equal numbers of hermaphrodites and neuters. The frequencies of these hermaphrodites and neuters is expected to be determined by the recombination rate between the male- and female-sterility loci (Charlesworth and Charlesworth 1978).

Alternatively, female-sterility factors may only cause partial sterility and may result in slight shifts in the relative allocation to pollen and ovule output. When modifiers of fruit production are adjusting fruit set, neuters would not be expected to segregate from crosses between males and females because there would be no factors conferring complete female sterility. Because no neuters were found in progeny from crosses which produced hermaphrodites and neuters were very rare in nature, this suggests that female-sterility mutations do not cause complete loss of the production of fruit. Rather, it is further evidence supporting the contention that genetic modifiers are causing slight shifts in the allocation to male and female reproductive output.

The only other subdioecious species for which genetic factors modifying fruit set in male-fertile individuals have been found is *Hebe subalpina* (Delph and Lloyd 1991). In *H. subalpina*, the genotype of the clone accounted for a significant proportion of the variance in fruit production of male-fertile individuals (Delph and Lloyd 1991). Delph and Lloyd (1991) hypothesized that gene expression in *H. subalpina* was controlled by seed-reduction modifiers which act by raising a resource threshold over which the individual will produce seeds. The data from experiments with *A. bitteriata* suggest that a resource-based mechanism does not control seed production because production of seeds was not associated with size of corm the year of flowering. However, corm size may not
be the best indicator of resources available for fruit production.

Hermaphrodites with reduced stamen production have also been reported in three gynodioecious species, *Origanum vulgare* (Kheyr-Pour 1980), *Silene vulgaris* (Horovitz and Dulberger 1983, Dulberger and Horovitz 1984) and *Plantago lanceolata* (Van Damme and Van Delden 1984, de Haan 1996). Although inheritance of reduced stamen production in *P. lanceolata* is complex and involves interactions between cytoplasmic and nuclear factors (Van Damme 1983, de Haan 1996), inheritance in *S. vulgaris* may be much simpler. In *S. vulgaris* segregation of genotypes with reduced stamen production was accounted for by a model of inheritance of sex expression whereby females were *Aaaa* or *aaaa*, hermaphrodites with complete stamens were *AAAA* and *AAAa* and hermaphrodites with reduced stamen production were *AaAa* (Horovitz and Dulberger 1983).

No strong relationship between sex-expression and environment was detected in this study. However, the experiments on the effects of environment and size presented here should not be considered as conclusive because sample sizes were extremely small and the high and low light and nutrient environments may not mimic the environmental variation seen in nature. It would not be surprising to find a relationship between fruit set and size or environment in future studies of male-fertile individuals because allocational shifts which alter fruit production in response to environment, size, or defoliation appear to be common in hermaphroditic plants (Delph 1993, Klinkhamer and de Jong 1997).

**Field Sex Ratios** - Sex ratios in different *Astilbe biternata* populations were quite variable and often deviated from equal frequencies of male-fertiles and females.
Similar high variation in sex ratios has been found in other subdioecious species (Barrett 1992, Wolfe and Shmida 1997). In addition, A. biternata sex ratios are more commonly male-fertile than female biased. This is a general trend among subdioecious and many gynodioecious species (Ganders 1978, Webb 1979, Delph 1990c, Barrett 1992, Wolfe and Shmida 1997, for exception see Hermanutz and Innes 1994).

Theoretical expectations for sex ratio frequencies of subdioecious species are not well developed, especially if cyto-nuclear associations or sex ratio distorsers are influencing sex-expression (Frank 1989, Charlesworth 1981, Gouyon et al. 1991, Schultz 1994). Nonetheless, striking negative correlations between the frequency of females and the frequency of male-fertiles which produce seeds have been found in two subdioecious species (Barrett 1992, Wolfe and Shmida 1997). The theory behind these associations is well developed for gynodioecious species with nuclear inheritance of male-sterility at equilibrium (Lloyd 1976). However, no association between female frequencies and frequencies of seed-producing male-fertiles was found in populations of A. biternata sampled in 1994 or 1995. Several factors may account for this discrepancy, among them are the following. First, cyto-nuclear genetic factors may determine sex expression. In such cases, cyclic changes in sex ratios through time are possible (Gouyon et al. 1991, Schultz 1994) and sex ratios at any given time may or may not be related to the seed contribution of females and hermaphrodites. Second, because A. biternata is long-lived, possibly reaching ages over 20 years, at any one time sex ratios may represent the overlap of several generations of individuals. In this case, present sex ratios may not be reflected by relative reproductive output of females and hermaphrodites. Last, because male-
fertiles produce seeds in some years and not in others, environmental factors may also affect the relative ratios of hermaphrodites to male-fertiles.

In conclusion, the reproductive system of *Aristolochia biternata* appears to lie along the continuum between gynodioecy and dioecy. Several lines of evidence including gender transitions between years in the same individuals, patterns of the distributions of quantitative phenotypic gender, and sex ratios in natural populations support this view. There also is genetic variation for both propensity to produce fruit as well as for stamen production in male-fertiles suggesting that the reproductive system may be in evolutionary flux. However, it cannot yet be determined whether the breeding system is evolving toward hermaphroditism or towards dioecy or it represents an equilibrium state. The evolutionary trajectory of subdioecy in *A. biternata* is assessed in Chapter 3 by comparing the relative fitnesses of males, females, and hermaphrodites.

The results of experimental crosses suggest that the inheritance of sex-expression in *A. biternata* may be quite complex, possibly involving cytoplasmic and nuclear genes or sex ratio distorters. Because the genetic basis for sex-determination has been shown to have profound effects on the evolution of subdioecious reproductive systems (Charlesworth and Charlesworth 1978, Maurice *et al.* 1993, 1994, Schultz 1994, Nordborg 1994), a definitive assessment of the evolutionary status of the breeding system in *A. biternata* will require that an understanding of the genetic basis of sex-expression be developed concurrently with studies of the fitnesses of the different genders.
Table 2.1. Sex ratios in *Astillbe bibernata* populations in 1994. Refer to Figure 2.2 for locations of populations. Chi-square goodness of fit tests performed on the null hypothesis of 1:1 ratios of females: males+hermaphrodites; ** = P<0.0025 *=0.0025<P<0.05.

<table>
<thead>
<tr>
<th>Population</th>
<th>females (%)</th>
<th>males (%)</th>
<th>hermaphrodites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tate 2</td>
<td>30 (61)</td>
<td>17 (35)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Haywood</td>
<td>70 (48)</td>
<td>66 (46)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Shope Fork</td>
<td>138 (46)</td>
<td>131 (48)</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Ball Creek</td>
<td>144 (41)</td>
<td>124 (48)</td>
<td>32 (11)</td>
</tr>
<tr>
<td>Standing Indian</td>
<td>83 (39)</td>
<td>114 (54)</td>
<td>14 (6) **</td>
</tr>
<tr>
<td>Flattop</td>
<td>30 (38)</td>
<td>49 (61)</td>
<td>1 (1) *</td>
</tr>
<tr>
<td>US 74</td>
<td>81 (38)</td>
<td>103 (48)</td>
<td>31 (14) **</td>
</tr>
<tr>
<td>Pearson</td>
<td>39 (36)</td>
<td>64 (59)</td>
<td>5 (5) **</td>
</tr>
<tr>
<td>Alarka 2</td>
<td>11 (35)</td>
<td>20 (65)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tate 1</td>
<td>12 (33)</td>
<td>21 (58)</td>
<td>3 (8) *</td>
</tr>
<tr>
<td>US 80</td>
<td>29 (31)</td>
<td>45 (48)</td>
<td>20 (21) **</td>
</tr>
<tr>
<td>Alarka 1</td>
<td>20 (30)</td>
<td>44 (66)</td>
<td>3 (4) **</td>
</tr>
<tr>
<td>US 215</td>
<td>22 (28)</td>
<td>49 (64)</td>
<td>6 (8) **</td>
</tr>
<tr>
<td>226a</td>
<td>29 (27)</td>
<td>55 (51)</td>
<td>24 (22) **</td>
</tr>
<tr>
<td>Highway 9</td>
<td>17 (25)</td>
<td>40 (58)</td>
<td>12 (18) **</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>748 (40)</strong></td>
<td><strong>949 (51)</strong></td>
<td><strong>178 (9)</strong></td>
</tr>
</tbody>
</table>
Table 2.2. Sex ratios in *Astillbe bittersata* populations in 1995. Refer to Figure 2.3 for locations of populations. Chi-square goodness of fit test performed on the null hypothesis of 1:1 ratios of females: males+hermaphrodites: ** = P<0.0025 *=0.0025<P<0.05.

<table>
<thead>
<tr>
<th>Population</th>
<th>females (%)</th>
<th>males (%)</th>
<th>hermaphrodites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tate 2</td>
<td>7 (78)</td>
<td>1 (11)</td>
<td>1(11)</td>
</tr>
<tr>
<td>Shake Rag</td>
<td>5 (56)</td>
<td>3 (33)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Shope Fork</td>
<td>28 (54)</td>
<td>221 (42)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Dickinson</td>
<td>9 (50)</td>
<td>9 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Haywood</td>
<td>49 (48)</td>
<td>49 (48)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Wise</td>
<td>25 (47)</td>
<td>18 (34)</td>
<td>10 (19)</td>
</tr>
<tr>
<td>US215</td>
<td>45 (45)</td>
<td>55 (55)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>US 80</td>
<td>28 (44)</td>
<td>31 (48)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Pearson</td>
<td>44 (44)</td>
<td>47 (47)</td>
<td>10(10)</td>
</tr>
<tr>
<td>US 74</td>
<td>48 (42)</td>
<td>46 (40)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>Standing Indian</td>
<td>43 (41)</td>
<td>60 (57)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Alarka 2</td>
<td>10 (40)</td>
<td>13 (52)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Ball Creek</td>
<td>44 (40)</td>
<td>61 (55)</td>
<td>5 (5) *</td>
</tr>
<tr>
<td>Clinch</td>
<td>18 (32)</td>
<td>34 (61)</td>
<td>4 (7) **</td>
</tr>
<tr>
<td>Tate 1</td>
<td>5 (31)</td>
<td>9 (56)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Cumberland</td>
<td>15 (30)</td>
<td>21 (42)</td>
<td>14 (28) *</td>
</tr>
<tr>
<td>Pound Gap</td>
<td>27 (29)</td>
<td>63 (68)</td>
<td>2 (2) **</td>
</tr>
<tr>
<td>226a</td>
<td>20 (23)</td>
<td>53 (62)</td>
<td>13 (15) **</td>
</tr>
<tr>
<td>Alarka 1</td>
<td>3 (14)</td>
<td>15 (71)</td>
<td>3 (14) **</td>
</tr>
<tr>
<td>Fork Mountain</td>
<td>11 (17)</td>
<td>36 (55)</td>
<td>18 (28) **</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>484 (39)</td>
<td>646 (51)</td>
<td>121 (10)</td>
</tr>
</tbody>
</table>
Table 2.3. Sex ratios of *Aristolba biternata* populations at Ball Creek and Shope Fork between 1994 and 1997. Goodness-of-fit tests were performed to test the null hypothesis of equal numbers of male-fertile (males + hermaphrodites) and female individuals. * = P < 0.05, ** = P < 0.01.

**BALL CREEK**

<table>
<thead>
<tr>
<th>Year</th>
<th>Female (%)</th>
<th>Male (%)</th>
<th>Hermaphrodite (%)</th>
<th>Neuter (%)</th>
<th>$\chi^2$ (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>144 (48.0)</td>
<td>124 (41.3)</td>
<td>32 (11.7)</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>1995</td>
<td>44 (39.6)</td>
<td>61 (55.0)</td>
<td>5 (4.5)</td>
<td>1 (0.9)</td>
<td>4.4*</td>
</tr>
<tr>
<td>1996</td>
<td>72 (55.0)</td>
<td>34 (26.0)</td>
<td>25 (19.0)</td>
<td>0</td>
<td>1.29</td>
</tr>
<tr>
<td>1997</td>
<td>102 (44.9)</td>
<td>84 (37.0)</td>
<td>39 (17.2)</td>
<td>2 (0.9)</td>
<td>1.96</td>
</tr>
</tbody>
</table>

**SHOPE FORK**

<table>
<thead>
<tr>
<th>Year</th>
<th>Female (%)</th>
<th>Male (%)</th>
<th>Hermaphrodite (%)</th>
<th>Neuter (%)</th>
<th>$\chi^2$ (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>131 (46.0)</td>
<td>138 (48.4)</td>
<td>16 (5.6)</td>
<td>0</td>
<td>1.86</td>
</tr>
<tr>
<td>1995</td>
<td>28 (52.8)</td>
<td>22 (41.5)</td>
<td>3 (5.7)</td>
<td>0</td>
<td>0.17</td>
</tr>
<tr>
<td>1996</td>
<td>29 (41.4)</td>
<td>36 (51.4)</td>
<td>5 (7.2)</td>
<td>0</td>
<td>2.06</td>
</tr>
<tr>
<td>1997</td>
<td>68 (39.5)</td>
<td>100 (58.2)</td>
<td>4 (2.3)</td>
<td>0</td>
<td>7.53**</td>
</tr>
</tbody>
</table>
Table 2.4. Flowering frequency of *Aristolochia biflora* males, females, and hermaphrodites that first flowered in 1994. Plants were followed for two years at Ball Creek and three years at Shope Fork. For this table, males were individuals that never produced seeds in any year in which they flowered but produced pollen in every year in which they flowered. Hermaphrodites were individuals which produced both pollen and seeds in at least one year in which they flowered.

<table>
<thead>
<tr>
<th>BALL CREEK</th>
<th>Number flowering at least “x” years after 1994</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (%)</td>
<td>Females (%)</td>
</tr>
<tr>
<td>x=0</td>
<td>79 (84%)</td>
<td>84 (82)</td>
</tr>
<tr>
<td>x=1</td>
<td>15 (16)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>x=2</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SHOPE FORK</th>
<th>Number flowering at least “x” years after 1994</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (%)</td>
<td>Females (%)</td>
</tr>
<tr>
<td>x=0</td>
<td>82 (67%)</td>
<td>82 (71)</td>
</tr>
<tr>
<td>x=1</td>
<td>40 (33)</td>
<td>33 (29)</td>
</tr>
<tr>
<td>x=2</td>
<td>11 (9)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>x=3</td>
<td>1 (1)</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2.5. ANOVA table testing for differences between the numbers of seeds per capsule borne on females and hermaphrodites. Population, population*Sex and Replicate (Pop*Sex) were treated as random variables. Because the experiment was unbalanced, denominator mean squares and degrees of freedom for F-tests were calculated using the SAS statistical package (SAS Institute 1993). Population*Sex was the main component in the denominator mean square when testing for differences in seeds per capsule between females and hermaphrodites. SS refers to type III sums of squares.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>4</td>
<td>222.4</td>
<td>1.13</td>
<td>0.4556</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>92.4</td>
<td>1.92</td>
<td>0.2255</td>
</tr>
<tr>
<td>Population*Sex</td>
<td>4</td>
<td>197.5</td>
<td>1.14</td>
<td>0.3562</td>
</tr>
<tr>
<td>Replicate (Pop*Sex)</td>
<td>33</td>
<td>1460.1</td>
<td>5.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>185</td>
<td>1395.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.6. ANOVA table of differences in the inflorescence lengths of males, females and hermaphrodites of *Astrilbe biternata* at Ball Creek and Shope Fork (Site treatment) in 1995 and 1996. All treatments were considered fixed effects. SS refers to type III sums of squares.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>1</td>
<td>73.36</td>
<td>0.58</td>
<td>0.4465</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>202.86</td>
<td>1.61</td>
<td>0.2059</td>
</tr>
<tr>
<td>Sex</td>
<td>2</td>
<td>429.39</td>
<td>1.70</td>
<td>0.1842</td>
</tr>
<tr>
<td>Pop*Year</td>
<td>1</td>
<td>516.68</td>
<td>4.09</td>
<td>0.0439</td>
</tr>
<tr>
<td>Pop*Sex</td>
<td>2</td>
<td>229.05</td>
<td>0.91</td>
<td>0.4047</td>
</tr>
<tr>
<td>Year*Sex</td>
<td>2</td>
<td>296.75</td>
<td>1.17</td>
<td>0.3101</td>
</tr>
<tr>
<td>Pop<em>Year</em>Sex</td>
<td>2</td>
<td>197.88</td>
<td>0.78</td>
<td>0.4576</td>
</tr>
<tr>
<td>Error</td>
<td>348</td>
<td>43948.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7. ANOVA table of relationship between the length of the inflorescence and the log_{10}(flower number) for male, female, and hermaphroditic *Astilbe biternata*. When sexes are pooled the curvilinear relationship between height and flower number is significant at \( P<0.02 \). SS refers to type III sums of squares.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>2</td>
<td>0.1028</td>
<td>1.46</td>
<td>0.2388</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>0.4610</td>
<td>13.13</td>
<td>0.0006</td>
</tr>
<tr>
<td>Sex*Height</td>
<td>2</td>
<td>0.0746</td>
<td>1.06</td>
<td>0.3516</td>
</tr>
<tr>
<td>Height*Height</td>
<td>1</td>
<td>0.1042</td>
<td>2.97</td>
<td>0.0897</td>
</tr>
<tr>
<td>Sex<em>Height</em>Height</td>
<td>2</td>
<td>0.0545</td>
<td>0.78</td>
<td>0.4648</td>
</tr>
<tr>
<td>Error</td>
<td>65</td>
<td>2.2830</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.8. Numbers of *Astillbe biterrnata* clones that did and did not set fruit for five male-fertile genotypes. n.a. = not applicable.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>100</th>
<th>102</th>
<th>121</th>
<th>730</th>
<th>781</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of clones that did not set fruit</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Number of clones that set fruit</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Average percent fruit set for clones that set fruit</td>
<td>n.a.</td>
<td>48.3</td>
<td>n.a.</td>
<td>27.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Table 2.9. Results from seven crosses in 1995 between male-fertile and female individuals of *Aristolb bitenata*. Hermaphrodites had either full or reduced stamen production. Crosses were done in three populations US74, Ball Creek, and Shope Fork. Percentages of each sex within the cross are given in parentheses. The sex of progeny was recorded in 1997. $\chi^2$ refers to goodness of fit tests testing the null hypothesis of equal numbers of females and males + hermaphrodites. * = 0.05>P>0.005, ** = P<0.005. † Note that plant 121 at US74 is not the same individual as 121 in Table 8.

<table>
<thead>
<tr>
<th>Population</th>
<th>Cross</th>
<th>Male</th>
<th>Female</th>
<th>Hermaphrodite</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Full Stamen Production</td>
<td>Reduced Stamen Production</td>
</tr>
<tr>
<td>US74</td>
<td>100σ×104♀</td>
<td>12 (52)</td>
<td>11 (48)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>123σ×103♀</td>
<td>25 (78)</td>
<td>1 (3)</td>
<td>5 (16)</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td>120σ×121♀ †</td>
<td>3 (27)</td>
<td>7 (46)</td>
<td>3 (27)</td>
<td>0</td>
</tr>
<tr>
<td>Ball Creek</td>
<td>501σ×170♀</td>
<td>3 (8)</td>
<td>29 (78)</td>
<td>1 (3)</td>
<td>4 (11)</td>
</tr>
<tr>
<td></td>
<td>501σ×787♀</td>
<td>2 (4)</td>
<td>35 (65)</td>
<td>8 (15)</td>
<td>9 (16)</td>
</tr>
<tr>
<td></td>
<td>502σ×787♀</td>
<td>11 (35)</td>
<td>19 (61)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>106σ×105♀</td>
<td>4 (9)</td>
<td>26 (58)</td>
<td>15 (33)</td>
<td>0</td>
</tr>
<tr>
<td>Shope Fork</td>
<td>781σ×210♀</td>
<td>19 (49)</td>
<td>19 (49)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2.1. Male, female, and hermaphrodite flowers of *Astillbe biternata*. A. Receptive female flowers. Note the absence of stamens. B. Male-fertile flowers at anthesis. Note 10 stamens per androecium and extension of filaments beyond the sepals. C. Male-fertile flowers with reduced numbers of stamens at anthesis. Note that many flowers have less than 10 stamens which are often not fully exerted. D. Male-fertile flowers with developing fruit. Note enlarged fruit on some flowers but not on others. Dry stamens are still visible on many flowers.
Figure 2.2. Sex ratios of 15 *Astilbe biternata* populations in 1994. All but two populations were located in the southern Appalachian Mountains of North Carolina. The Tate 1 and Tate 2 populations were located in northern Georgia. The counties in which *A. biternata* has been found are indicated in green. The county distribution was first developed by Mellichamp (1976) and amended during the field studies described here. Counties in the southeastern corner of the distribution are not shown in full because this is the southeastern edge of the blue ridge and is a natural boundary for the range of *A. biternata*. 
Figure 2.3. Sex ratios of 20 *Aristolba biternata* populations in 1995. Populations were chosen to represent areas throughout the range of *A. biternata*. The counties in which *A. biternata* has been found are indicated in green. The county distribution was first developed by Mellichamp (1976) and amended during the field studies described here. Counties in the southeastern corner of the distribution are not shown in full because this is the southeastern edge of the blue ridge and is a natural boundary for the range of *A. biternata*. 
Figure 2.4. The relationships between the frequency of females and the frequency of male-fertiles which produced seed (hermaphrodites) in 1994 and 1995. Points on the graph represent the populations which were sampled in 1994 and 1995 and are the same populations as in Figures 2 and 3. For 1994 $P>0.20$, for 1995 $P>0.30$. 
Figure 2.5. Relationship between percent fruit set and percent stamen production in hermaphrodites of *A. biternata* at Ball Creek in 1997. Spearman’s Rho = -0.76, *P*<0.0001.
Figure 2.6. Estimates of standardized phenotypic gender for *A. biennis* individuals flowering in 1997 at Ball Creek and Shope Fork. Calculation of phenotypic genders is summarized in the methods.
Figure 2.7. All gender transitions for *A. biternata* witnessed at Ball Creek and Shope Fork between 1994 and 1997. M=male gender expressed, F=female gender expressed, H=hermaphrodite gender expressed.
### Shope Fork

<table>
<thead>
<tr>
<th>Initial Gender</th>
<th>H</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>11</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>54</td>
</tr>
</tbody>
</table>

### Ball Creek

<table>
<thead>
<tr>
<th>Initial Gender</th>
<th>H</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>13</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>8</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>61</td>
</tr>
</tbody>
</table>
Figure 2.8. Weights of corms which expressed male, female, or hermaphroditic genders in 1996. Corms were weighed during the early spring of 1997. Individual clones were grown in high light/high nutrient or low light/low nutrient environments. No difference in sex-expression between environments was detected.
Figure 2.9. Weights of male-fertile corms which did and did not produce fruit in the 1997 clonal repeatability studies. Corms represented clones from 3 genotypes 781, 730, and 102. All corms were grown in the same environment (see Methods). Corms were weighed during the early spring of 1997.
CHAPTER 3

Factors affecting the evolution of subdioecy in *Aristolochia biternata*
INTRODUCTION

The occurrence of a relatively high frequency of plant species in which different individuals express male, female, and hermaphroditic genders (termed subdioecy) remains a mystery to evolutionary biologists. In a 1922 survey of over 95,000 dicotyledon taxa, species with some combination of males, females, and hermaphroditic (or monoecious) individuals were found to be almost as common as dioecious species (Yamplonsky and Yamplonsky 1922). Despite these findings, researchers persist in viewing subdioecious reproductive systems as unusual (see Dormée et al. 1990). One reason for this view may be that early models of the evolution between hermaphroditism and dioecy did not adequately explain the theoretical conditions which would result in maintenance of subdioecy (Charnov et al. 1976). Because of this difficulty, subdioecy is often thought to be a transitional breeding system between hermaphroditism and dioecy and thus “uncommon” because of its ephemeral nature.

More recent theoretical models are able to predict stable subdioecy under three general scenarios: 1) when genetic linkage constraints are incorporated (Charlesworth and Charlesworth 1978), 2) when male sterility is determined by cyto-nuclear associations (Gregorius and Ross 1987, Maurice et al. 1993, 1994, Schultz 1994, Nordborg 1994), or 3) in the presence of pollinator limitation (Maurice and Fleming 1995). “Genetic linkage constraints” refers to linkage between male and female sterility factors in the nuclear genome (Charlesworth and Charlesworth 1978) where male and female sterility are determined at two different loci. Under this scenario, males carry alleles for male fertility and female sterility (at separate loci) and females carry alleles for male sterility and
female fertility (at separate loci). Without complete linkage disequilibrium between the male and female sterility factors crosses, crosses between males and females will bring together male and female fertility factors when recombination occurs. Thus, hermaphrodites (male and female fertiles) and neuters (male and female steriles) will segregate from crosses between males and females.

Linkage constraints are also important if male sterility is determined by cytoplasmic factors (Maurice et al. 1993, Schultz 1994). For dioecy to evolve there must be complete linkage between male fertility restorers (which result in male fertility in the presence of certain cytoplasmic male sterility factors) and female sterility alleles, both of which are located in the nuclear genome. In the presence of a cytoplasmic male sterility factor, recombination between these nuclear factors results in female fertiles which are restored for male fertility (hermaphrodites) and female steriles that are not restored (neuters). Whether male sterility is determined by nuclear or cytoplasmic factors, linkage constraints result in maintenance of subdioecy when unisexuals (males and females) both have higher total fitness than hermaphrodites (Charlesworth and Charlesworth 1978, Maurice et al. 1993, Schultz 1994). Thus, males and females are present in populations because they have greater fitness than hermaphrodites, and hermaphrodites are present because they are continually being reintroduced into the population by recombination between the nuclear male and female sterility factors (Charlesworth and Charlesworth 1978).

More than one cytoplasmic male sterility type can also persist in a subdioecious population under certain conditions. For example, in theoretical models assuming one
"normal" male fertile cytoplasm and a second cytoplasm causing male-sterility, hermaphrodites are maintained in subdioecious breeding systems only when both the sterility and non-sterility cytoplasm persist in the population; otherwise, dioecy evolves (Maurice et al. 1993, Nordborg 1994). The non-sterility cytoplasm is maintained when the female sterility factor decreased the female fertility of individuals with the male-sterility cytoplasm (Maurice et al. 1993). When both cytoplasmic factors are maintained, subdioecy persists only when males have higher outcross siring success than hermaphrodites and females either have higher seed fitness than hermaphrodites or there are costs of restoration of male-sterility (Maurice et al. 1993, Nordborg 1994). This model has been expanded to include two male-sterility cytoplasms and the general predictions for maintenance of subdioecy remain the same as for models with one male sterile cytoplasm (Maurice et al. 1994, Schultz 1994). However, caution must be used when interpreting the evolutionary trajectory of a subdioecious species when sex-determination involves both nuclear and cytoplasmic factors because the outcome of selection cannot always be predicted by the number of successful gametes produced by each sex morph (Maurice et al. 1993). Although increased siring success of males is necessary for the persistence of males in subdioecious populations, it is not a sufficient condition for the persistence of males (Maurice et al. 1993). Persistence of males also depends on complex dynamics between the male-sterility cytoplasms and the nuclear restorers which cannot be predicted by the siring success of males (Maurice et al. 1993).

Theoretical models predict that subdioecy can also be a stable breeding system when the level of pollen limitation is dependent on the frequency of females in the
population (Maurice and Fleming 1995). Basically, hermaphrodites persist because as female frequencies increase, pollinator limitation becomes more severe thus decreasing the number of seeds on females which are pollinated; hermaphrodites are able to assure reproduction because they can produce self-pollinated seeds. Like the two previous cases, subdioecy is stable only when males have higher outcross siring success than hermaphrodites and females produce more fruit than hermaphrodites (Maurice and Fleming 1995).

Although these models differ in the mechanisms which allow subdioecy to persist, they all predict that for subdioecy to evolve from gynodioecious ancestors, females are expected to have higher seed set than hermaphrodites and males are expected to have higher outcross siring success than hermaphrodites. If these conditions are not met, the breeding system will evolve to either gynodioecy or dioecy.

There are only four subdioecious species for which reproductive success of the different sex types has been determined in sufficient detail to test the predictions of theoretical models (Opuntia robusta, Del Castillo 1986; Schiedea globosa, Sakai and Weller 1991; Wurmbea dioica, Barrett 1992; and Pachycereus pringlei, Fleming et al. 1994). In the absence of pollinator limitation, theory would not predict the maintenance of males in three of these four cases (Pachycereus pringlei, Schiedea globosa, and Wurmbea dioica), because males do not produce sufficiently more pollen than hermaphrodites and are not expected to sire more seeds (but see Maurice and Fleming 1995 regarding P. pringlei). This inconsistency between theoretical predictions and empirical observations may be a result of an inadequate appraisal of the selfing rates of
hermaphrodites (in some cases selfing rates were not measured) and inadequate appraisal of the relative outcross siring success of males and hermaphrodites. These studies concentrated on indirect estimates of outcross siring success such as pollen production and flower number, both of which may not accurately reflect differences in reproductive success through pollen (Meagher 1991). In this chapter, I present the results of direct measurements of siring success and selfing rates from paternity analyses within experimental arrays. Direct measurements are currently the best way to make accurate measurements of siring success and selfing rates (Stanton et al. 1992, Snow and Lewis 1993).

This chapter focuses on comparisons of the relative fitnesses of males, females, and hermaphrodites in *Astilbe b坚韧ata*, a subdioecious species (see Chapter 1). Here I measure fitness-related factors considered theoretically important for the persistence of subdioecy and ask the following questions of relevance in theoretical models: 1) do females produce more seeds than hermaphrodites?, 2) do males have higher outcross siring success than hermaphrodites?, and 3) are selfing rates and inbreeding depression sufficiently high that they reduce the fitness of seeds produced by hermaphrodites relative to seeds produced by females?

**METHODS**

Estimates of individual fitness in *Astilbe b坚韧ata* are complicated by its perennial habit as well as by the large number of flowers (therefore the large number of seeds and anthers) produced on inflorescences. For this study, flowering frequency of the
genders was compared to determine whether fitness could vary as a result of increased flowering frequencies for some genders relative to others. Inflorescence length was used to estimate the number of flowers produced by each individual and relative seed set and stamen production were determined from estimates of total fruit and stamen set per inflorescence. The numbers of seeds per fruit were also compared for females and hermaphrodites. For hermaphrodites, inbreeding depression was estimated in greenhouse studies, and selfing rates were estimated in experimental arrays. Finally, siring success of male-fertiles was estimated within experimental arrays.

Theoretical models assume that male-fertile individuals are fixed in their gender expression: in other words, males never produce seeds and hermaphrodites always produce seeds in every year in which they flower. From previous studies it is clear that this assumption does not hold for *A. biternata*. Male-fertiles are able to express both hermaphroditic (produce seeds & pollen) and male genders (produce pollen only) in different years (see Chapter 2). However, the propensity to produce seeds in male-fertile individuals is determined in part by the genotype of the individual (Chapter 2). When I assigned gender to the male-fertiles in the experimental arrays to estimate siring success, the assumption was made that the gender expression (male or hermaphrodite) of a male-fertile in the field in 1994 (when they were collected) was correlated with the genotype (i.e. "hermaphrodite" = male-fertile with a high propensity to produce fruit or "male" =male-fertile with a low propensity to produce fruit). Therefore, without subjecting each individual to a clonal repeatability experiment, assigning a genotype to a male-fertile individual remains tentative.
COMPARISONS OF FLOWERING FREQUENCY - Flowering frequency of males, females, and hermaphrodites was compared by analysis of census records from two populations, Shope Fork and Ball Creek, at Coweeta Hydrological Lab. At Shope Fork all plants that flowered in 1994 were marked with flags and scored for the number of times they flowered over the next 3 years. At Ball Creek only the next two years of data were used because in 1997 many flags had been removed by logging operations and most flowering individuals could not be re-identified. For this analysis, any plant which had a hermaphroditic gender in at least one flowering season was considered to be a hermaphrodite. A goodness of fit test (Steel and Torrie 1980) was used to test the null hypothesis that the proportion of individuals that flowered at least once subsequent to the initial census was independent of gender.

REPRODUCTIVE OUTPUT - ESTIMATION OF FRUIT SET AND STAMEN PRODUCTION - Because A. biternata inflorescences contain thousands of flowers, determining the exact fruit set and stamen production for hundreds of individuals in situ was infeasible. Therefore, the following methods were developed to obtain quick and accurate estimates of the percent fruit set and percent stamen production for each individual.

In 1995 fruit set of hermaphrodites and females was compared in 11 populations covering the range of A. biternata. Each inflorescence was assigned a percent fruit set to the nearest 10% based on a visual assessment of the proportion of the number of flowers which developed fruit. Percent fruit set was assigned to one of the 13 following categories: 0, 2, 10, 20, 30, 40, 50, 60, 70, 80, 90, 98, or 100%. At Coweeta, inflorescences with between 10 and 90% fruit set were revisited to check the accuracy of
initial assessments. In other populations only a subset of the individuals (approximately 10 individuals) were revisited to check the accuracy of initial estimates.

At the Ball Creek and Shope Fork populations the relative stamen production was also assessed for all male-fertile individuals. Male-fertile *A. biternata* flowers produce at most 10 stamens each. In some male-fertile individuals stamen production per flower varies from 0 to 10 stamens. An appraisal which was analogous to that for percent fruit set was developed for percent stamen production. Each inflorescence was assigned a percent stamen production to the nearest 10% based on a visual assessment of the proportion of the stamens produced on the entire inflorescence (relative to the maximum of 10 stamens per flower). Percent stamen production was assigned to one of the 13 following categories: 0, 2, 10, 20, 30, 40, 50, 60, 70, 80, 90, 98, or 100%. Inflorescences with between 10% and 90% stamen production were revisited to check the accuracy of initial assessments.

*Data analysis* - Non-parametric Kruskal-Wallis statistics generated from PROC NPAR1WAY in the SAS statistical package (SAS Institute 1993) were used to test for differences in percent fruit set and stamen production among individuals expressing different genders during the year in which they were sampled. Additionally, non-parametric correlation analyses of stamen and fruit production were calculated with JMP (Sas Institute 1995) and I report Spearman's Rho, a correlation coefficient computed on the ranks of the data.

*Numbers of seeds per fruit* - The fruits (capsules) of *A. biternata* are composed of two locules. The number of seeds per fruit was estimated by counting the number of
seeds in one randomly selected locule from five fruits on five females and five hermaphrodites from the US74, Ball Creek, Shope Fork, and 266a populations. Additionally seeds per locule were counted on five fruits from two females and two hermaphrodites from the US80 population. The null hypothesis of no difference in the numbers of seeds per locule for females and hermaphrodites was tested using a mixed model analysis of variance.

**INBREEDING DEPRESSION**—The relative germination rates and seedling growth rates of self-fertilized and outcross-fertilized progeny of hermaphrodites were compared in growth chamber and greenhouse environments to estimate inbreeding depression. Both self and outcross pollinations were performed three hermaphrodites in the greenhouse in 1995. One of these hermaphrodites (102) was grown from seed collected at the Ball Creek population, one (214) was collected as a corm from the Ball creek population, and the third (38) was collected as a corm from the Shope Fork population. Up to five male-fertile genotypes contributed outcross pollen to each of the three hermaphrodites. All of the outcross pollen parents originated from the Ball Creek population. To prevent unwanted pollen from reaching stigmas, all flowers on small inflorescence stems (with approximately 25-30 flowers each) were bagged with cellulose tubing and flowers were emasculated prior to anther dehiscence. Flowers on separate stems of the inflorescence were self- or outcross-pollinated. Pollinations from each outcross genotype was restricted to a different stem of the inflorescence.

Self and outcross pollinations were completed in June of 1996 and mature seeds were collected in September. In November, between 150 and 200 seeds from each sire by
dam combination were planted in Scotts Metro-Mix 220 soil mixture and placed under grow lights in a growth chamber at a constant temperature of 22°C. Germination rates were monitored every other day. Six weeks after germination all seedlings were transplanted to 4 cm diameter conetainers and transferred to the greenhouse where the temperature was kept above 15°C and the plants were fertilized every other week.

As seedlings grow they produce new leaves which originate from a small developing corm at the soil surface and old leaves senesce. The petiole on each successive leaf is longer than the petiole on the preceding leaf. The petioles continue to increase in size through the first two years of growth in the greenhouse. To quantify variation in growth rates among selfed and outcrossed progeny, the height of the longest petiole on each of the selfed plants and on 25 outcrossed plants from each sire by dam outcross combination was measured in late February 1997. Information on relative inflorescence size, relative flower production, and time to first reproduction is not included here because *A. biternata* requires a minimum of 2 years to grow from seed to flowering and the selfed and outcrossed progeny had not flowered by the time of this report.

**ESTIMATION OF OUTCROSS SIRING SUCCESS AND SELFING RATES IN EXPERIMENTAL ARRAYS - DESIGN OF ARRAYS** - Outcross siring success (of both males and hermaphrodites) and selfing rates of hermaphrodites were estimated by creating experimental arrays wherein males and hermaphrodites had different known allozyme genotypes. In these experimental arrays, the sex of male-fertile individuals (males or hermaphrodites) was defined by whether the individual had produced seeds in the field in
1994. I first screened the genotypes of males, females, and hermaphrodites from the Shope Fork, Ball Creek, and US74 populations at 2 allozyme loci, PGI and PGM. PGI and PGM loci were unlinked in A. biernata (see Chapter 4). Individuals were chosen for placement in arrays to maximize the possibility of discriminating between progeny from males and hermaphrodites using these two allozyme loci. In most cases some discrimination among potential fathers within the male and hermaphrodite classes was possible. Because individuals which expressed hermaphroditic genders were relatively rare compared to males and allozyme genotypes varied more between populations than within populations, I was not able to take all individuals from one population. Seven of the ten hermaphrodites used in the arrays originated from the US 74 population, two hermaphrodites came from Shope Fork, and all other males, females, and hermaphrodites originated from the Ball Creek population. To ensure that the results were not biased by particular alleles being genetically linked with traits conferring increased siring ability, different PGI and PGM alleles were used to discriminate among males and hermaphrodites in different arrays. Outcross siring success was estimated using seeds from females within the arrays, while selfing rates were estimated using seeds from hermaphrodites.

Four experimental arrays were constructed at Coweeta hydrological laboratory in 1996. Each array was placed in a different watershed each of which was at least ½ mile from any natural population of A. biernata. One array (Weir) was placed at the edge of a field while the other three arrays were placed in the forest understory. Long distance pollination could be detected, if present, in the two arrays which were in closest
proximity to one another (approximately 300 m apart separated by a ridge thickly covered with rhododendron). Each array had individuals with allozyme genotypes not found in the other array. Because all allozyme genotypes of progeny from these two arrays could be accounted for by pollen donors within the array, long-distance pollination was assumed to be negligible.

Arrays were designed to include three males, three hermaphrodites, and six females in a spatially-symmetrical design such that there was both a male and a hermaphrodite at each of three distances from a given female (Figure 3.1). In 1996, Cove 2 was the only array in which all individuals flowered and all females set fruit (Figure 3.1). If male or hermaphrodite individuals did not flower they were either replaced as soon as their non-flowering state was apparent, or if no individual with the appropriate gender and allozyme genotype was available, both the non-flowering individual and the pollen-donor with which it was paired (either male or hermaphrodite) were removed from the array.

Experimental arrays were established in mid-May. At this time, potted plants were placed in holes and each plant was surrounded by an open-top 1" mesh chicken wire cage to deter deer herbivory. *Astillbe biternata* is pollinated by a wide array of small flying insects, primarily small bees and flies. Observations of pollinator foraging verified that flower access to pollinators was not restricted by the cages. Every few days during the flowering period from early June to early July each array was visited and the proportion of flowers with dehisced anthers and/or with receptive stigmas were estimated for each plant. Stigmas were designated as receptive when they appeared moist. Stigmas which
were dry on the surface were assumed to be non-receptive.

In mid-July, after all flowers on all plants were well past anthesis, the plants were moved to the Botany Greenhouses at Duke University. After inflorescences senesced, fruits were collected and the number of flowers per inflorescence was counted.

For each female, all seeds were separated from fruits, mixed, and stored in envelopes. A random sample of seeds was planted in soil and grown under standard fluorescent lights in a growth room at a constant temperature of 22°C until plants were sufficiently large to yield leaf samples for allozyme analysis (see Chapter 4 for methods). Up to 120 progeny from each female in the array (and all offspring from females that produced fewer than 120 seeds) were scored for allozyme genotypes. The bilocus genotypes of these progeny were used to estimate the outcross siring success of all male-fertiles in experimental arrays using maximum likelihood algorithms (see below).

DATA ANALYSIS - Because *Astillbe biternata* is a tetraploid and inheritance of allozyme markers is disomic (see Chapter 4), when there were three different alleles at one locus (e.g. 1223) or two copies of each of two alleles (e.g. 2233) at the same locus, associations between alleles within subgenomes for each individual in each array had to be determined to estimate segregation ratios of allozyme markers for each individual. For six cases associations were determined by conducting experimental crosses (see methods and analysis in Chapter 4). For an additional three plants, allele associations were determined by analyzing the genotypes of the possible progeny and all other potential parents assuming no contamination from plants outside the arrays. Sample sizes within the arrays were sufficiently large that the associations determined for the latter three
plants can be accepted with reasonable confidence. Genotypes and segregation ratios of allosemic markers were assigned to all individuals in each experimental array.

**Maximum likelihood estimation of outcross siring success and selfing**

When estimating paternity it is best to have each potential sire associated with a unique allele, then paternity assessment is unambiguous. If this is not possible, as in this study, it is best to maximize the number of alleles or combinations of alleles which are unique to one potential sire. The next best alternative is to have alleles or combinations of alleles which are unique to a subset of all possible sires. When all potential sires do not possess unique alleles, maximum likelihood estimation of paternity has been shown to be the most accurate method (Devlin et al. 1988, Smouse and Meagher 1994). In this study maximum likelihood methods were used to estimate both the relative siring success for each male-fertile individual in each array as well as the selfing rate for the hermaphrodite which set seed.

Outcross siring success, $\theta$, was defined as the relativized proportion of seeds sired by a particular male-fertile individual (male or hermaphrodite) on a particular female and was estimated using the seed genotype data obtained from each female. The non-selfed progeny from hermaphrodites were not used to estimate outcross siring success because only one hermaphrodite set seed. Thus, for the data from each female within each array, up to six $\theta$ parameters were estimated, one for each pollen producing plant in the array. If a male-fertile individual was not flowering at the same time as a female, it was left out of the analysis.

The selfing rates of the hermaphrodite was estimated using seed from the
hermaphrodite; otherwise, all estimation procedures discussed below were the same.

Selfing rate was assigned as the proportion of seeds ($\theta$) which were sired by the
hermaphrodite which produced the seeds. Only 1 hermaphrodite set sufficient seed (31
progeny were scored for PGI and PGM loci) to estimate a selfing rate.

The probabilities ($P_{ijk}$) of each sire $i$ yielding an offspring with genotype $j$ when
mating with dam $k$ were enumerated assuming disomic inheritance and no linkage of PGI
and PGM loci (see Chapter 4). For each offspring genotype, $P_{ijk}$ was multiplied by the
siring ability parameters $\theta_{ik}$ for each potential sire $i$ on each dam $k$ to yield the expected
fraction of progeny of genotype $j$ among the offspring of dam $k$. Assuming the
distribution of the progeny array genotypes is multinomial, the likelihood ($\mathcal{L}$) of
observing the particular distribution of offspring genotypes from each dam $k$ given the
probability model is:

$$\mathcal{L} = C \prod_{i,j} [(P_{ijk} \times \theta_{ik})^{n_{jk}}]$$

where $C$ is the multinomial coefficient and $n_{jk}$ is the number of seeds with genotype $j$
from each dam $k$. Because the multinomial coefficient is independent of the siring ability
parameters, it was not included in the estimation procedure.

The maximum likelihood for the siring success parameters was determined by
computing the negative natural log of the likelihood (i.e. $-\ln(\mathcal{L}) = -\sum_{i,j} (n_{jk} \times \ln(P_{ijk} \times \theta_{ik}))$)
and finding the combination of $\theta_{ik}$'s which minimized the $-\ln(\mathcal{L})$. Minima were found
using the FindMinimum command in Mathematica 2.1 (Wolfram 1991). FindMinimum
finds only local minima, therefore a thorough search of the parameter space (of $\theta_{ik}$'s)
using various combinations of starting conditions was necessary to determine a global minimum. The search of the parameter space was restricted so that $\sum_{i,k} \theta_{ik} < 1$ and numerous combinations of $\theta_{ik}$'s which systematically searched the parameter space by changes of 0.1 were used. This procedure was repeated for each dam in each array.

When siring ability is estimated relative to all the other potential sires (as in this study), under the null hypothesis of equal siring abilities among all sires, the expected value for all $\theta_{ik}$'s is $\frac{1}{n}$, where $n$ is the number of potential sires. Therefore, the estimate of siring success ($\theta_{ik}$) is contingent on the number of sires in the array. To adjust for this effect, all $\theta_{ik}$'s were standardized by dividing $\theta_{ik}$ by $\frac{1}{n}$; thus, under the null hypothesis, a siring ability equal to the null expectation was given a value of 1.0.

*Hypothesis testing* - The standardized maximum likelihood estimates of outcross siring success ($\theta_{ik}$'s) for each possible sire for each female were used as the dependent variable in an analysis of covariance designed to test the effect of the sex of the sire (i.e. male or hermaphrodite) on its siring ability. Standardized siring abilities ($\theta_{ik}$'s) were log transformed before analysis to normalize the distribution of residuals. The data were analyzed as a split-plot with SEX (male or hermaphrodite) and ARRAY in the main plot and FATHER(ARRAY*SEX) in the sub-plot. SEX was considered a fixed effect while ARRAY, SEX*ARRAY, and FATHER(ARRAY*SEX) were treated as random effects. Because the analysis was unbalanced, approximations of the appropriate denominator mean squares and degrees of freedom were used to test for differences in siring success between sexes. These estimates were calculated and incorporated into F-tests using the JMP statistical package (SAS Institute 1995).
Two covariates, OVERLAP (in flowering time) and the DISTANCE between potential sires and a female in meters, were incorporated into the above split plot analysis. OVERLAP was computed as the proportion of overlap in flowering dates between a specific sire and the mother, relative to the other prospective sires in the population. For example, if only two potential sires were flowering at the same time as a female, if sire A’s flowering period overlapped five out of ten days with the flowering female and sire B overlapped ten out of ten days, sire A would be assigned an OVERLAP of 5/15=1/3 and sire B would be assigned an OVERLAP of 10/15=2/3. Because OVERLAP was a proportion, it was arcsin square-root transformed to normalize the distribution of residuals prior to analysis (Steel and Torrie 1980). DISTANCE was entered into the model untransformed.

For the analysis of covariance the null hypotheses of homogeneity of regression slopes between all treatments and the individual covariates were not rejected at $\alpha=0.05$. In addition, the test of homogeneity of the regression plane between all treatments and both covariates was not rejected at $\alpha=0.05$. Therefore, the regression plane and individual regression slopes were assumed not to interact with the treatments (Huijema 1980). Likewise, Bartlett’s test for homogeneity of variance was not significant for all treatments.

To explore relationships between the siring success and flower number, the mean siring success adjusted for OVERLAP and DISTANCE was regressed on the relative flower number of each potential sire within each array.
RESULTS

FREQUENCY OF FLOWERING - Flowering frequencies were low for all three sexes (Table 3.1). Overall only 32% of plants which flowered in 1994 flowered at least once more over the subsequent 3 years at Shope Fork, and only 18% of plants which flowered in 1994 flowered again over the next 2 years at Ball Creek. For plants which flowered during the 1994 growing season, there was no statistical difference among males, females, and hermaphrodites in the frequency of plants that flowered at least once more in subsequent years (Table 3.1; Shope Fork, Fisher's exact test P=0.14; Ball Creek, Fisher's exact test P=0.75).

REPRODUCTIVE OUTPUT - TRADEOFF BETWEEN STAMEN AND FRUIT PRODUCTION - During 1997 in the Ball Creek population, both percent stamen production and percent fruit set were measured to investigate tradeoffs between stamen and fruit production. Average stamen production of hermaphrodites at Ball creek was 25% lower than the average stamen production of males (Kruskal-Wallis Chi-square approximation = 39.2, df=1, P<0.0001; sample sizes: n_hermaphrodites = 38, n_males = 75). In the same population, average percent fruit set was 66.3% lower in hermaphrodites than females. As shown in Figure 3.2 there was a significant negative correlation between percent stamen production and percent fruit set on individual hermaphrodites in this population (Spearman's Rho = -0.76, P<0.0001), suggesting a tradeoff in allocation to male and female reproductive output in hermaphrodites.

SEED SET - The relationship between the length of the inflorescence and the log of the number of flowers per inflorescence was quadratic curvilinear and did not vary among
males, females, and hermaphrodites (Figure 3.3; Table 3.2; y = 2.10936+0.03957x-
0.00022x^2, R^2=0.70). Therefore, females and hermaphrodites were assumed to have the
same relationship between the number of flowers produced and the length of the
inflorescence.

Within sites in which 10 or more hermaphrodites flowered in 1995 (Pearson,
US74, Wise and 226A populations), there was a significant difference in the lengths of
inflorescences produced by males, females, and hermaphrodites (Table 3.3). Pairwise
contrasts revealed that hermaphrodite inflorescences were significantly shorter than male
and female inflorescences (Figure 3.4). Conversion of the average length of inflorescence
into the average flower production (using regression equations) estimated that
hermaphrodites produce 24.4% fewer flowers than females.

In 1995, fruit set was estimated for 104 hermaphrodites and 271 females in a
eleven populations throughout the range of A. biternata (Figure 3.5). Overall, females
averaged 3.75 times higher fruit set than hermaphrodites (85.2% fruit set for females
versus 22.7% fruit set for hermaphrodites). Females had significantly higher average fruit
set than hermaphrodites (blocking on population, Friedman's test = 142.8, df=1,
P<0.001). The number of seeds per fruit did not differ significantly between females and
hermaphrodites (Table 3.4; for females 11.02±0.71, hermaphrodites 9.62±0.70).

Overall, hermaphrodites produced an estimated 9,601 seeds on average ( = #
flowers * % fruit set * 2 locules/fruit * seeds/locule = 2014 * 0.227 * 2 * 10.5) while
females produced 47,682 seeds on average ( = # flowers * % fruit set * 2 locules/fruit *
seeds/locule = 2665 * 0.852 * 2 * 10.5). Thus, overall females produced an estimated
4.97 times as many seeds as hermaphrodites.

**FLOWER NUMBERS ON MALES AND HERMAPHRODITES** - To estimate the difference in the numbers of flowers produced by males and hermaphrodites, the expected number of flowers was computed using the mean inflorescence lengths for male and hermaphrodites and the regression equations for the relationships between flower number and inflorescence lengths (see above). Hermaphrodite inflorescences were 18% shorter than male inflorescences on average (Figure 3.4; Table 3.3). Males with average sized inflorescences are estimated to produce 2996 flowers and hermaphrodites of average size were estimated to produce 2014 flowers; males therefore produced 32.8% more flowers than hermaphrodites.

**INBREEDING DEPRESSION** - Germination rates of seeds varied among hermaphrodites (Figure 3.6, see Dam x Cross type term in Table 3.5). Although selfed progeny had lower germination rates than outcrossed progeny for each hermaphrodite, the differences were only significant for progeny from one hermaphrodite. There was a 55% reduction in germination rate for selfed seeds from plant 214 compared to outcrossed seeds (LSD t = 5.78, P< 0.001; Figure 3.6), but no significant difference between selfed and outcrossed seed germination rates for the other two hermaphrodites. Overall, the reduction in germination rates resulting from inbreeding depression was 0.18.

For all families, progeny from self fertilizations were significantly smaller after four months than progeny from outcross fertilizations (Table 3.6). Progeny from outcross-pollinations averaged 7.0 cm while progeny from self-pollinations averaged 5.95 cm. Although the size of seedlings is difficult to interpret in terms of its effect on fitness, it
does suggest a general reduction in vigor among inbred progeny compared to outcrossed progeny which may foreshadow fitness reductions later in life.

**SELFING RATE OF HERMAPHRODITE** - The one hermaphrodite in the 1996 experimental populations that produced seeds had an estimated selfing rate of 0.85.

**EXPERIMENTAL ESTIMATION OF OUTCROSS SIRING SUCCESS** - Of the two covariates (OVERLAP and DISTANCE) only overlap in flowering time explained a significant proportion of the variation in siring success (OVERLAP; Table 3.7).

There was no overall effect of the sex of the sire on outcross siring success after adjusting for the overlap in flowering time and distance covariates (hereafter termed "adjusted siring success"; Table 3.7). The effect of sex on siring varied among arrays (SEX*ARRAY interaction, see Table 3.7, Figure 3.7). Pairwise contrasts between male and hermaphrodite adjusted siring success within each array showed that in Cove 1, hermaphrodites sired significantly fewer seeds than males (LSD t ratio = 3.43, P<0.005; see Figure 3.7). However, I suspect that the large difference in siring between males and hermaphrodites in Cove 1 array may have been an anomalous result because one hermaphrodite in this array was extremely small and unhealthy to the point that its inflorescence senesced during the flowering period. In all other arrays males and hermaphrodites did not sire significantly different proportions of outcrossed seeds after adjusting for flowering overlap and distance (in all arrays LSD P>0.05; Figure 3.7).

Along with the hermaphrodite in Cove 1, one other male fertile individual senesced during the experiment, a male in Cove 2. Both of these individuals sired fewer seeds than the male-fertile individual with which they were paired in the array. If these
two pairs of individuals are excluded, 8 pairs of males and hermaphrodites remain. Of these eight, in all but one pair the male sired more seeds than the hermaphrodite after adjusting for distance and flowering phenology. Under the null hypothesis of equal siring success four pairs are expected to have higher siring success for hermaphrodites and four are expected to have higher siring success for males. Applying the sign test, the probability of seven of the eight pairs with males siring more seeds than hermaphrodites is 0.035.

Siring success and flower number - Overall, no relationship between the relative flower number of a plant and its siring success was detected after adjusting for flowering overlap and distance ($F_{1,18}=1.06$, $P>0.30$).

DISCUSSION

Relative reproductive output - Females and hermaphrodites- Under any of the theoretical scenarios proposed for the maintenance of females in gynodioecious and subdioecious species, Astilbe biternata females will persist in populations because they produce over four times as many seeds as hermaphrodites. This is well over the minimum doubling of seed production which is theoretically necessary for females to persist in populations with hermaphrodites when male sterility is determined by nuclear genes and there is no inbreeding depression and selfing (Charlesworth and Charlesworth 1978, 1981). As inbreeding depression and selfing rates of hermaphrodites increase, the relative amount by which a female must out-reproduce hermaphrodites in order to reproduce decreases further (Charlesworth and Charlesworth 1978). If male-sterility is
determined by cytoplasmic factors the differential seed output required for female persistence in even smaller (Lewis 1941, Charlesworth 1981, Gouyon et al. 1991). Therefore, regardless of the genetic system the breeding system in *A. biternata* is unlikely to lose females.

The estimates of relative seed production for females and hermaphrodites in *A. biternata* are comparable to what has been found for other gynodioecious and subdioecious species. For example subdioecious *Ochradenus baccatus* (Wolfe and Shmida 1997) and *Schiedea globosa* (Sakai and Weller 1991) females produce about five times as many fruits or seeds as hermaphrodites; subdioecious *Pachycereus pringlei* (Freeman et al. 1994) and *Opuntia robusta* (Del Castillo 1986) females produce about 1.6 times as much fruit as hermaphrodites produce; and, gynodioecious *Silene acaulis* (Morris and Doak, in press) for which females are expected to produce 4.4 times as many seeds as hermaphrodites over their lifetimes.

For some gynodioecious and subdioecious species it has been postulated that the differences in fruit set between females and hermaphrodites can be explained in part through the process of reproductive compensation (Ashman 1994, Wolfe and Shmida 1997). The concept of reproductive compensation was first introduced by Darwin (1877) and states that when resource allocation to one sex is reduced, the unused resources can be re-allocated into increasing the reproductive output of the other sex (Charnov 1982). Although it is not known whether the patterns in relative fruit production arose because of reproductive compensation, the striking negative correlation between fruit set and stamen production within hermaphrodites (Figure 3.2) suggests that there may be a tradeoff
between allocation to male and female reproductive output in *A. biternata* hermaphrodites. Similar negative correlations between male and female reproductive output in hermaphrodites have been found in two other subdioecious species, *Pachycereus pringlei* (Freeman *et al.* 1994) and *Ochradenus baccatus* (Wolfe and Shmida 1997), but were not detectable in *Schiedea globosa* (Sakai and Weller 1991). However, for no subdioecious species is it known how the tradeoffs in reproductive output translate into reproductive success.

**Siring success** - Before discussing the results of the siring success experiments I reiterate that male-fertile *A. biternata* individuals are able to express both hermaphroditic (produce seeds & pollen) and male genders (produce pollen only) in different years (see Chapter 2). Moreover, the propensity to produce seeds in male-fertile individuals is determined in part by the genotype of the individual (Chapter 2). When I assigned gender to the male-fertiles in the experimental arrays to estimate siring success, I made the assumption that the gender expression (male or hermaphrodite) of a male-fertile in the field in 1994 (when they were collected) was correlated with the genotype (i.e. "hermaphrodite" = male-fertile with a high propensity to produce fruit or "male" = male-fertile with a low propensity to produce fruit). Therefore, without subjecting each individual to a clonal repeatability experiment, assigning a genotype to a male-fertile individual remains tentative.

In Cove 1, one hermaphrodite senesced before fruit and seed developed. Because there was only one other hermaphrodite in the population, I excluded the results from the entire array for some analyses. If the results from the Cove 1 array are excluded, there
were no general differences between the siring success of hermaphrodites and males after adjusting for flowering overlap and distance. However, two plants were not healthy during the experiment. If the two pairs of sires (male & hermaphrodite) which included these two plants are excluded, in seven of the eight remaining pairs, males sired more seeds than hermaphrodites, after adjusting for overlap and distance. This result is consistent with predictions from theoretical models that subdioecy should persist only in cases where males sire more seeds than hermaphrodites (Charlesworth and Charlesworth 1978, Maurice et al. 1993, Schultz 1994). Although this analysis was done post facto and may therefore be biased, it is nonetheless worthwhile to report that seven of eight males sired more seeds that the hermaphrodite with whom they were paired.

In the experimental arrays only one hermaphrodite (out of 10) produced a sufficient number of seeds to estimate its selfing rate and outcross siring success. Therefore, the results from this study show that even in years when hermaphrodites do not set seed, they have slightly lower siring success than males. Because seed production in hermaphrodites is inversely correlated with stamen production, hermaphrodites may have even lower siring success in years in which they produce seeds (Figure 3.2). Therefore, understanding the relationship between siring success and the tradeoff between fruit production and stamen production may also be necessary in order to understand the evolution of subdioecy in A. biternata.

*Flower number*- Surprisingly, there was no correlation between the number of flowers produced by male-fertile individuals (males+hermaphrodites) and their outcross siring success in the experimental arrays. A relationship between flower number and
siring success was also absent for *Chamaelirium luteum* (Meagher 1991), but has been found for *Asclepias exaltata* (Broyles and Wyatt 1990) and *Raphanus raphanistrum* (Devlin *et al.* 1992). In *C. luteum* most of the variation in siring success could be accounted for by the distance between males and females (Meagher 1991). Distance effects in the experimental arrays were not detectable, but the length of overlap in flowering times had a strong effect on siring success.

In natural populations males produce more than 30% more flowers than hermaphrodites. The results from this experiment suggest that simply the difference in flower production *per se* does not result in differential siring abilities between males and hermaphrodites. Rather, other factors, possibly nectar production, flower presentation, or “attractiveness” of the inflorescence to pollinators, which are not related to flower production, are resulting in increased siring success among males compared to hermaphrodites.

**SELFING RATES AND INBREEDING DEPRESSION IN HERMAPHRODITES** - In addition to relative seed set and siring success, inbreeding depression is expected to play an important role in the evolution from hermaphroditism to dioecy in many systems (Charlesworth and Charlesworth 1978, 1981). Because increased selfing rates magnify the effects of inbreeding depression on progeny from hermaphrodites, the effects of inbreeding depression in natural populations cannot be determined without also estimating selfing rates. Although the data presented in this study remain tentative because inbreeding was measured from crosses on only three hermaphrodites from two different populations and the selfing rate has to be inferred from seeds produced by one
hermaphrodite, the presence of potentially high selfing rates and moderate inbreeding depression suggests these factors merit further study.

*Inbreeding depression* - The effect of inbreeding depression varied among families in *Aristolba biternata*. Although selfed progeny from all families had lower germination rates than outcrossed progeny, there was a significant effect of inbreeding depression on germination rate in only one family. In this family, selfing reduced the germination rate by over 50%. Similar variation for inbreeding depression has been found in other subdioecious species (*Schiedea globosa*, Sakai et al. 1989; *Hebe subalpina*, Delph and Lloyd 1996) as well as numerous other plants. In *S. globosa*, some families showed no inbreeding depression, while in other families inbreeding depression was more than 0.5 (Sakai et al. 1989). Variation in the extent of inbreeding depression among families is expected if the hermaphrodites vary in their histories of inbreeding or numbers of deleterious alleles (Haldane 1949, Johnston and Schoen 1994).

Inbreeding depression also affected the growth rates of seedlings. Selfed seedlings had slower growth rates than outcrossed seedlings in all families. Although it is not yet clear what effect the growth rate of seedlings has on fitness, it is possible that reduction in growth rate may slow the time to first reproduction or it may have implications for the ability to compete for space when new sites are colonized. This study reports only on the effects of inbreeding depression during the early stages of the life cycle, whereas in inbred lineages deleterious recessive alleles which affect early stages of the life cycle are expected to be purged before those that affect later stages (Husband and Schemske 1997). As the progeny from the inbreeding experiments mature, they may exhibit stronger effects.
of inbreeding depression.

Inbreeding depression in *A. biternata* was measured in growth chamber and greenhouse environments. Inbreeding depression measured in controlled environments is generally thought to underestimate inbreeding depression in natural situations where environmental fluctuations and biotic interactions are important (Schoen 1983, Schemske 1983, Dudash 1990, Schmitt and Ehrhardt 1990, Eckert and Barrett 1994). Because accurate estimates of inbreeding depression are essential for assessing the evolutionary trajectory of the breeding system, future studies should consider estimating inbreeding depression in the field as well as the greenhouse.

**Selfing rates of hermaphrodites** - In *A. biternata* the selfing rate for the only hermaphrodite to set seed in the experimental arrays was greater than 0.80. Although the general level of selfing in *A. biternata* hermaphrodites cannot be determined from one individual in one setting, selfing rates can, nevertheless, be potentially high in some cases. When selfing rates are high, conditions are created where inbreeding depression will affect the majority of the seeds produced by hermaphrodites and could have large effects on decreasing the fitness of seeds produced by hermaphrodites relative to seeds produced by females.

**The Evolution of Subdioecy** - Understanding the evolution of subdioecious species requires a knowledge of both the relative fitnesses of males, females, and hermaphrodites and the genetics underlying sex expression. Here I present data which is qualitatively consistent with theoretical predictions of the relative fitnesses of males, females, and hermaphrodites when subdioecy persists in these models. However, I have
not yet considered whether the magnitude of the differences between the relative fitnesses of male, females, and hermaphrodites is consistent with theoretical predictions.

Theoretically, the magnitude of the differences in the relative fitnesses necessary for persistence of subdioecy is contingent on the underlying genetics of sex-expression (Charlesworth and Charlesworth 1978, Maurice et al. 1993, 1994, Schultz 1994). However, the genetic basis for inheritance of sex-expression in not yet clear in A. biternata (Chapter 2). Nevertheless, it may be informative to assess the relative contributions of males, females, and hermaphrodites in the framework of an analytical model of resource allocation using the ESS approach (Charlesworth and Charlesworth 1981, see Maynard Smith 1976). Although this model does not account for the persistence of subdioecy, it does predict the relative fitnesses necessary for males and females to increase in frequency when introduced into hermaphroditic populations assuming nuclear inheritance of genes determining sex-expression.

Charlesworth and Charlesworth's (1981) ESS model predicts that females will increase in hermaphroditic populations when:

\[ k > 1 - 2s\delta \]

where \( k \) is the increase in seed production of females relative to hermaphrodites, \( s \) is selfing rate of hermaphrodites, and \( \delta \) is the inbreeding depression (Charlesworth and Charlesworth 1981). Even if inbreeding depression were not present, females would be predicted to persist in hermaphroditic populations because they produce well over twice the number of seeds that hermaphrodites produce.
Males will increase in frequency (in gynodioecious populations) only if

\[ K > \frac{1 + s(1 - 2\delta)}{k - s(1 - 2\delta)} \]

where \( K \) represents the relative increase in outcross siring success over hermaphrodites, and \( s, \delta, \) and \( k \) are as above (Charlesworth and Charlesworth 1981). Using the estimates of \( k = 4.97, \delta = 0.18, \) and \( s = 0.85 \) for \textit{A. biternata}, males would have to increase outcross siring success by 35\% compared to hermaphrodites to persist in populations. Even in the scenario most beneficial for males (taking the average siring success of males compared to hermaphrodites), males would be expected to sire only 24\% more outcrossed seeds than hermaphrodites. Thus, according to this model and these estimated values, males are predicted to be lost from populations in the future.

This is the first study which has brought together estimates of male and female fitness, inbreeding depression, and selfing rates for \textit{A. biternata} (or any other species), and some of these estimates remain tentative. The estimates of inbreeding depression of hermaphrodites, the relative seed production of females compared to hermaphrodites, or the relative outcross siring success of males compared to hermaphrodites reported here may be underestimates. Increases in any of these factors would aid in the maintenance of males in populations. There is reason to believe that inbreeding depression is an underestimate (see above). Furthermore, if hermaphrodites do not set fruit every year in which they flower, the disparity in lifetime seed production of females and hermaphrodites may be even larger than estimated in this study (though probably not dramatically - compare to estimate in Chapter 2 of relative fruit set over several years at
Ball Creek and Shope Fork). Also, most of the hermaphrodites in the experiments reported here did not set seed. As more data for estimation of the relative outcross siring success of males and hermaphrodites which set seed is gathered, hermaphrodites which set seed may prove to have lower outcross siring success than both males and hermaphrodites which do not set seed.

Cyto-nuclear inheritance of sex-expression can also theoretically aid the maintenance of males in subdioecious populations (Maurice et al. 1994). However, the genetics of sex-expression are not yet clear in A. biternata (see Chapter 2). Because the qualitative estimates of the relative fitnesses of females and hermaphrodites are consistent with theoretical predictions, future studies should focus on understanding of the influence of the mode of inheritance of male-sterility on the evolution of subdioecy in A. biternata.

Finally, if pollinator limitation is dependent on the frequency of females in the population, it may account for the persistence of subdioecy in some cases (Maurice and Fleming 1995). The relationship between pollinator limitation and female frequency has not been estimated for A. biternata. The power of pollinator limitation to stabilize subdioecy is greatest when selfing rates of hermaphrodites are high (Maurice and Fleming 1995), which may be the case for A. biternata. Because qualitative differences in the fitnesses of males, females, and hermaphrodites are consistent with this model, future studies of the effects of pollinator limitation should be considered.
Table 3.1. Flowering frequency of *Aristolba biternata* males, females, and hermaphrodites sampled in 1994. Flowering was recorded for two years at Ball Creek and three years at Shope Fork. Percentages in parentheses indicate the proportion of individuals of each sex which flowered *at least* "x" times during the subsequent years are given in parentheses. Therefore, the percentages do not sum to 100%.

<table>
<thead>
<tr>
<th></th>
<th>BALL CREEK</th>
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<td></td>
<td>Number flowing at least &quot;x&quot; times in 1995 and 1996</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>x=0</td>
<td>79 (84%)</td>
<td>84 (82)</td>
<td>21 (78)</td>
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<td>15 (16%)</td>
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<th>SHOPE FORK</th>
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<td>Number flowing at least &quot;x&quot; times in 1995 and 1997</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>x=0</td>
<td>82 (67%)</td>
<td>82 (71)</td>
<td>8 (47)</td>
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<tr>
<td>x=3</td>
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Table 3.2. A) ANOVA table of the quadratic curvilinear relationship between the length of the inflorescence and the log_{10}(flower number) for *Astilbe bibernata*. B) ANOVA table comparing the curvilinear relationship among males, females and hermaphrodites. SS refers to type III sums of squares.

### A.

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</tr>
<tr>
<td>Error</td>
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### B.

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Table 3.3. ANOVA table testing for differences between lengths of inflorescences produced by males, females, and hermaphrodites in four populations in 1995. The length of the inflorescence was log transformed prior to analysis. Because the experiment was unbalanced, denominator mean squares and degrees of freedom for F-tests were calculated using the JMP statistical package (SAS Institute 1995). Population*Sex was the main component in the denominator mean square when testing for differences in the height of inflorescences among males, females, and hermaphrodites.

<table>
<thead>
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<td>Sex</td>
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<td>5.94</td>
<td>0.0348</td>
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<tr>
<td>Population*Sex</td>
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<td>Error</td>
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Table 3.4. ANOVA table testing for differences between the numbers of seeds per capsule borne on females and hermaphrodites. Population, population*Sex and Replicate (Pop*Sex) were treated as random variables. Because the experiment was unbalanced, denominator mean squares and degrees of freedom for F-tests were calculated using the JMP statistical package (SAS Institute 1995). Population*Sex was the main component in the denominator mean square when testing for differences in seeds per capsule between females and hermaphrodites.

<table>
<thead>
<tr>
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</table>
Table 3.5. ANOVA table for tests of differences between germination rates of selfed and outcrossed seeds. The dependent variable, percent germination, was arcsin square-root transformed prior to analysis. Denominator mean squares and degrees of freedom for F-tests were calculated using the JMP statistical package (SAS Institute 1995) and are adjusted for the unbalanced design assuming that Dam, Dam*Cross type, and Sire(Dam*Type) are random effects.

<table>
<thead>
<tr>
<th>Source</th>
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Table 3.6. ANOVA table from tests of differences in height of petioles after four months from selfed and outcrossed seedlings. Denominator mean squares and degrees of freedom for F-tests were calculated using the JMP statistical package (SAS Institute 1995) and are adjusted for the unbalanced design assuming that Dam, Dam*Cross type, and Sire(Dam*Type) are random effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
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<td>Error</td>
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Table 3.7. ANOVA table testing the effects of sex (male or hermaphrodite) on outcross siring success. The dependent variable, standardized siring success ($\theta_{ab}$), was log transformed before analysis and Overlap was arcsin square-root transformed prior to analysis. Because the experiment was unbalanced, denominator mean squares and degrees of freedom for F-tests were calculated using JMP (SAS Institute 1995). Population*Sex was the main component in the denominator mean square when testing for differences in seeds per capsule between females and hermaphrodites. SS refers to Type III sums of squares.

<table>
<thead>
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<th>Source</th>
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<tr>
<td>Sire (Array*Sex)</td>
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<td>Overlap</td>
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<td>1.1202</td>
<td>33.55</td>
<td>0.0001</td>
</tr>
<tr>
<td>Distance</td>
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<tr>
<td>Error</td>
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Figure 3.1. Layout of experimental arrays at Coweeta Hydrological Laboratory in 1996. The Cove 1 Array was the only array in which all individuals flowered and all females set sufficient fruit for paternity analyses. Plants which did not flower are indicated by dashed lines.
Figure 3.2. Correlation of the percent fruit set and percent stamen production of *Aristolbe bitemnata* hermaphrodites at the Ball Creek population in 1997. Spearman’s Rho = -0.76, P<0.0001.
Figure 3.3. Mean percent fruit set of *Astillbe biternata* hermaphrodites (yellow histogram) and females (red histogram) in eleven populations in the southern Appalachian mountains of the United States in 1995. For all graphs, abscissas indicate the proportion of fruit set for females or hermaphrodites. The range of *A. biternata* is indicated in green on the map. State abbreviations are shown at the boundaries of the map.
Percent Fruit Set

- hermaphrodites
- females

1995
Figure 3.4. Quadratic curvilinear relationship between the length of the inflorescence and the flower number for *Astillbe biternata* males, females, and hermaphrodites. Note the log scale on the y-axis. Regression equation:

\[ y = 2.10936 + 0.03957x - 0.00022x^2, \quad R^2=0.70. \]
Figure 3.5. Mean inflorescence lengths for *Aristolba biternata* males, females and hermaphrodites combined from 4 populations which each had greater than 10 hermaphrodites flower in 1995. The inflorescence was measured from the axil of the lowest inflorescence branch to the tip of the inflorescence. Means were log transformed prior to analysis. Histograms represent back-transformed means of $\log_{10}$(inflorescence height) and thus are geometric means of the inflorescence heights. Standard errors around the mean are represented by error bars in each histogram.
Figure 3.6. Comparison of germination rates for self and outcross pollinated progeny from three *Aristolochia biternata* hermaphrodites (102, 214, and 38). Percent germination was arcsin square-root transformed prior to analysis. Error bars represent ± 1 standard error.
Figure 3.7. Comparison of siring success of *Astillbe biternata* males and hermaphrodites within each of the four experimental arrays in 1996. Histograms represent the mean siring success of males or hermaphrodites in the four arrays after adjusting for the flowering time overlap and distance covariates (termed least-squares means or lsmeans). Error bars represent ± 1 standard error.
LSMEAN (log$_{10}$ (per plant siring success +1))

Array

male

hermaphrodite
CHAPTER 4

Bayesian procedures for discriminating among hypotheses with discrete distributions: Inheritance in the tetraploid *Astilbe biternata*
ABSTRACT

Discrimination between disomic and tetrasomic inheritance aids in determining whether tetraploids originated by allotetraploidy or autotetraploidy, respectively. Past assessments of inheritance in tetraploids have used analyses whereby each inheritance hypothesis is tested independently. I present a Bayesian analysis which is appropriate for discriminating among several inheritance hypotheses, and can be used in any case where hypotheses are defined by discrete distributions. The Bayesian approach incorporates prior knowledge of the probability of occurrence of disomic and tetrasomic hypotheses so that the results of the analysis are not biased by the fact that there is a single tetrasomic hypothesis and multiple disomic hypotheses. This analysis is used to interpret data from crosses in the tetraploid *Aristolbe biternata*, a herbaceous plant native to the southern Appalachians. The progeny ratios from all crosses favored the hypothesis of disomic inheritance at both the *PGM* and *slow-PGI* loci. These results support earlier cytogenetic evidence for the allotetraploid origin of *Aristolbe biternata*. 

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INTRODUCTION

A principal concern in the study of genetics is the discrimination between models of inheritance for morphological or genetic traits. Often several hypotheses are constructed which may account for the inheritance of a trait. These hypotheses are then evaluated by screening progeny from crosses of individuals with known genotypes or phenotypes. Traditionally, goodness-of-fit analyses are used to discriminate among alternative hypotheses which may account for the observed pattern of segregation. Here I present a simple alternative statistical technique, Bayesian analysis, which I suggest is a more appropriate method for discriminating among alternative hypotheses.

Bayesian analysis is better suited to discrimination among alternative hypotheses than the goodness-of-fit analyses for the following three reasons. First, and most importantly, goodness-of-fit tests are limited to single contrasts between the null hypothesis and the distribution of the data. Hypotheses cannot be directly compared to one another. This can lead to two problems: 1) more than one hypothesis may be consistent with the data, so no decision can be made, and 2) the probability of type I error associated with multiple tests is inflated. In contrast, Bayesian analysis has the latitude to assess the confidence in a particular hypothesis relative to the entire set of hypotheses being considered. Second, the Bayesian approach can be used even when sample sizes are small (i.e. when sample sizes within cells are less than 5 or their average is less than 5), whereas chi-square approximations are less accurate when sample sizes are small (Steel and Torrie 1980). Third, a priori confidence in a hypothesis can be incorporated into the Bayesian approach. A priori confidence could be derived from knowledge of hierarchical
relationships among hypotheses (as in this paper), or information which a priori supports one hypothesis over others, such as phylogenetic relationships among species.

Herein the Bayesian and goodness-of-fit analyses are contrasted using the example of discriminating among several hypotheses for the inheritance of allozyme alleles in the tetraploid *Aristolbe biternata* (Saxifragaceae). Inheritance of markers from two allozyme systems, phosphoglucoisomerase and phosphoglucomutase, are analyzed to determine whether an autotetraploid or an allotetraploid origin is supported.

In general, four types of information are used to distinguish among different origins of tetraploids: 1) segregation patterns of genetic markers, 2) the presence or absence of multivalent formation, 3) tracing genetic markers from putative parental diploids to tetraploid derivatives, and 4) regeneration of tetraploids from the parental diploid(s) (Stebbins 1950). Several types of information are often combined before a firm conclusion is drawn because no single type of information is conclusive. For instance, the characterization of allele segregation in tetraploids as either disomic or tetrasomic is often very reliable for determining whether species have an autotetraploid or allotetraploid origin, respectively (Krebs and Hancock 1989). However, whereas tetrasomic inheritance is a good indicator of speciation via autotetraploidy, it is possible that a species with disomic inheritance may have originated via autotetraploid speciation and subsequently evolved disomic inheritance patterns at all chromosomes (see Sybenga 1969, Jackson and Jackson 1996). Likewise, multivalent formation is more common in autotetraploids than in allotetraploids. However, the presence or absence of multivalent formation is not a reliable method for discriminating among origins because autotetraploids do not always
exhibit multivalent formation (Soltis & Rieseberg 1986) and allotetraploids have been shown to form multivalents at times (Watson et al. 1991). In a few cases the allopolyploid origin of a species has been documented by observing genetic markers which are unique to each parental species and occur together in their allopolyploid derivative (e.g. Roose and Gottlieb 1976; Ashton and Abbott 1992; Wolf et al. 1990; Sun 1996). However, the reconstitution of tetraploids is often impractical because the parental species may no longer be extant (Werth and Lellinger 1992).

In general, the mode of origin of a tetraploid establishes the inheritance patterns of alleles. Autotetraploids arise when diploid gametes from a diploid parent (most commonly formed by gametic non-disjunction) fuse. Pairing of chromosomes at diplotene in autotetraploid species is completely random, or tetrasomic. Tetrasomic inheritance is characteristic of autotetraploids because the homeologous chromosome sets from the parents are structurally identical. Segregation in allotetraploids is quite different. Allotetraploids arise when hybridization between two dissimilar species is accompanied by doubling of the chromosome number (see Grant 1981). Disomic inheritance of alleles is characteristic of allotetraploids because the homeologous chromosome sets from the parental species are sufficiently different that one chromosome from each set always segregates into each gamete. Herein I will use the term “subgenome” to characterize alleles associated with the chromosomes from a particular parental species. In addition, species have been identified which are characterized by some loci segregating tetrasomically and others segregating disomically (de Vicente and Arús 1996). Termed segmental allotetraploids, these are hypothesized to have formed from the hybridization
of two closely related species such that the hybrid tetraploid has some identical homeologous chromosome sets and some dissimilar homeologous chromosome sets. Polyploidy has been estimated to account for between $\frac{1}{3}$ and $\frac{1}{2}$ of all extant Angiosperm taxa (Müntzig 1936; Darlington 1937; Stebbins 1971; Grant 1981). The origin of Angiosperm taxa via allopolyploidy is considered the most common mode of polyploid speciation (Grant 1981) and has been reported many times (e.g. Roose and Gottlieb 1976; Murdy and Carter 1985; Watson et al. 1991; Ashton and Abbott 1992; Sun 1996; Arulsekar et al. 1981; Hart 1983; Quiros and McHale 1985; Watson et al. 1991; Beaver and Iezzoni 1993). Although autopolyploidy is believed to be less common than allopolyploidy in Angiosperms (Grant 1981), observations of tetrasomic inheritance, a hallmark of autotetraploids, are becoming increasingly common (Soltis 1984; Martínez-Zapater and Oliver 1984; Hauber 1986; Krebs and Hancock 1989; Soltis and Soltis 1989; Cai and Chinnappa 1989). Moreover, it has been hypothesized that autopolyploid speciation may be a relatively common within some closely related plant taxa, in particular members of the Saxifragaceae (Soltis and Soltis 1989).

**STUDY SPECIES**

*Astilbe biternata* (Vent.) Britton is an herbaceous perennial in the Saxifragaceae family which is endemic to the Southern Appalachian Mountains. It typically grows in the shaded understory of rich mesic cove forests and along mesic roadsides. *Astilbe biternata* flowers in late June and early July and overwinters as a corm. Individuals can form large clumps of flowering ramets from a single corm. A large determinate inflorescence 20-80
cm long bears hundreds to thousands of flowers.

J. L. Hamel (1953) was the first to suggest that *Astillbe biternata* was an allotetraploid. His evidence was derived from a cytological investigation in which he found what appeared to be two divergent parental genomes within one of the 7 homeologous chromosome sets. Within one of the homeologous chromosome sets, which he called set b, one pair was isobrachiate and the other was heterobrachiate. The parental genomes were indistinguishable in all 6 of the other homeologous chromosome sets. Hamel used these observations to suggest that *Astillbe biternata* was an allotetraploid derived from the hybridization of two unknown divergent species (Hamel 1953). To date, no studies of multivalent formation have been attempted in *A. biternata*.

The genus Astilbe includes about 25 species, 23 of which occur in eastern Asia (Spongberg 1972). Recent molecular phylogenetic analyses place Astilbe + Saxifragopsis as sister to the Boykinia group + Leptarrhena-Tanakaeae clade within the Saxifragaceae *sensu stricto* (Soltis et al. 1996). The clade including Astilbe, Saxifragopsis, the Boykinia group, and Leptarrhena-Tanakaeae is sister to the clade including the Heuchera group where Heuchera and Tolmiea, two genera with autotetraploids (Soltis 1984; Soltis and Rieseberg 1986; Soltis and Soltis 1989, Ness et al. 1989; Wolf et al. 1990), reside.

**MATERIALS AND METHODS**

To assess inheritance of allozyme alleles in *Astillbe biternata*, four diagnostic crosses were performed in the greenhouses at Duke University, at Coweeta Hydrological laboratory, or at a site near U.S. Highway 74 outside Asheville, North Carolina.
Individuals for crosses were chosen so the ratios of progeny allozyme genotypes could be used to differentiate between disomic and tetrasomic inheritance. In all cases, peduncles of female inflorescences were bagged with cellulose tubing prior to the time when stigmas were receptive. Bags were removed only to pollinate the flowers. Whole flowers were removed from pollen donors and anthers were brushed several times across each receptive stigma to ensure pollination. To test whether foreign pollen could have entered when bags were removed, one bag was removed from the inflorescence on each female, the flowers were left unpollinated (pollinators were kept away), and the bag was replaced approximately 5 minutes later. No flowers on these inflorescences developed mature seeds.

Seeds from the crosses were collected and germinated on 1/4 Murashige & Skoog agar medium under florescent lights. Seedlings were planted into soil and grown until leaves were sufficiently large for enzyme extraction.

Fresh leaves were collected and stored at 4° until enzymes were extracted. Tris-HCl grinding buffer-PVP solution, 10% PVP (Soltis et al. 1983) was used to extract enzymes from leaves from all parents and progeny. Cellulose wicks were soaked in extract and frozen at -70° until electrophoresed on starch gels. The phosphoglucoisomerase loci (PGI) were resolved using system 6 gel and electrode buffers (Soltis et al. 1983) and the phosphoglocumutase locus (PGM) was resolved using system 9 gel and electrode buffers, pH 6.0 (Soltis et al. 1983). Standard staining procedures were used (Werth 1985; Wendel and Weeden 1989) to visualize enzyme systems. Allozyme alleles were numbered with the lowest number assigned to the most cathodally migrating
CALCULATION OF SEGREGATION RATIOS UNDER TETRASOMIC AND DISOMIC HYPOTHESES: Two types of tetrasomic segregation are often defined, chromosomal and chromatid segregation (Bever and Felber 1992). Chromosomal segregation refers to the case when the marker is sufficiently close to the centromere that crossovers between the centromere and the marker are rare, whereas chromatid segregation refers to the condition where the marker is far from the centromere and crossovers between the centromere and the marker are common. Under the chromosome model, segregation ratios are calculated as the possibility of drawing any pair from a set of 4 chromosomes (e.g. from 1223 parent, \(Pr(12) \text{ gamete} = 2/6\)). Whereas under the chromatid model with free recombination between the marker and the centromere, segregation ratios can be calculated as a random draw from the eight possible chromatids (e.g. from 1223 parent, \(Pr(12) \text{ gamete} = 2/8 \times 4/7 = 8/56\)). Under the chromatid model it is possible for two markers from sister chromatids to migrate into the same gamete (termed double reduction, see Bever and Felber 1992 for complete explanation). Because of gametes produced by double reduction, chromatid segregation can sometimes be recognized by the rare occurrence of genotypes which are not found under chromosomal segregation. For instance, in the cross represented in Table 4.1 (1223 x 2333) under a chromatid segregation model, six progeny genotypes are possible in low frequencies which are not possible under the chromosome segregation model (\(Pr(1122)=0.001, Pr(1123)=0.015, Pr(1133)=0.019, Pr(3333)=0.019, Pr(1222)=0.010, Pr(2222)=0.008\)). The presence of any of these genotypes indicates that multivalents have formed and double reduction has
occurred. In reality, chromosomal and chromatid segregation are endpoints in a continuum and most autotetraploids probably fall somewhere between these two extremes (Bever and Felber 1992, Jackson and Jackson 1996). The analysis presented here does not attempt to differentiate among intermediate types of tetrasomic segregation (see Jackson and Jackson 1996 for further discussion).

Disomic segregation occurs when there is complete fidelity in the pairing of specific chromosomes during diplotene. This has given rise to the term “subgenome” to differentiate the two set of chromosomes, which never mingle with one another during diplotene. There are usually multiple possible disomic hypotheses depending upon how the chromosomal markers are associated within the subgenomes. Calculation of the progeny frequencies under the two disomic hypotheses from the cross represented in Table 4.1 are diagramed in Figure 4.1. Under the “disomic 1” hypothesis markers 1_A and 2_A from plant 100 always pair at diplotene and markers 2_B and 3_B always pair at diplotene. The gametes produced from any particular meiotic event depend on the alignment of the two subgenomes (A and B) relative to one another. Because the two possible alignments are equally likely, four types of gametes are formed in equal proportions. Crossing the gametes from plant 100 and plant 104 result in the progeny frequencies under the disomic 1 hypothesis presented in Table 4.1. The disomic 2 hypothesis differs in that markers 1 and 3 both occur in subgenome A and both copies of marker 2 occur in subgenome B. Therefore only two types of gametes are formed in equal frequencies, and only four progeny genotypes are possible when plant 100 is crossed with plant 104 (see disomic 2 hypothesis in Table 4.1).
In this study I am primarily interested in whether segregation is tetrasomic or disomic and because no progeny were observed which appeared to be the products of double reduction, only chromosome segregation was considered. It is true that in most of the crosses in this study sample sizes were too small to reliably detect double reduction events; however, in most cases when no double reduction is observed, the results of the analysis are robust to including only the chromosome segregation model. This assumption is not valid in cases where the majority of double reduction events result in the same genotypes as under chromosome segregation models. This occurs in two cases: 1) 2333 x 2233, illustrated by the example presented in Table 4.2 and 2) when crossing two individuals each with two copies of two different markers (e.g. 2233 x 2233). In both of these cases, the progeny ratios under the chromatid segregation models will be more similar to a diploid segregation model (in Table 4.2 disomic hypothesis 2) than a tetrasomic chromosomal model and this could lead to a false interpretation! Therefore, it is important to obtain results from many crosses with different combinations of genotypes before a conclusion is drawn.

For each disomic and tetrasomic inheritance hypothesis, all possible progeny genotypes were enumerated and the expected proportions of progeny of each genotype were computed (see Figure 4.1 and Tables 4.1-4.6). Disomic hypotheses often had several alternatives depending on the possible pairing of alleles in the parental species. Because it was initially unclear whether the PGM alleles were associated with one or two loci, the expected proportions of progeny of each PGM genotype were also computed for several of the most plausible two-locus hypotheses (only the two most probable are shown in

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Table 4.5) for a cross which contained all alleles used in these analyses (123 x 103).

**Analysis Strategy:** Herein, Bayesian analysis (Schmitt 1969; Press 1989; Lee 1989) and goodness-of-fit analysis using a chi-square ($\chi^2$) test criterion (Steel and Torrie 1980, Zar 1984) are compared. Bayesian analysis differs from the goodness-of-fit analysis in two ways. First, Bayesian analysis can incorporate a discrete distribution (in this case the multinomial distribution) to determine the probability that an hypothesis is true given the data. The goodness-of-fit test relies on a continuous distribution, the chi-square distribution, which theoretically approximates the multinomial discrete distribution when the sample size is large (Zar 1984). This approximation biases the calculated $\chi^2$ value so the null hypothesis tends to be rejected with a probability greater than $\alpha$ (Zar 1984).

Second, they differ in their interpretation. Bayesian techniques can be considered to provide evidence *in favor* of a hypothesis, whereas the goodness-of-fit test is used to provide evidence *against* a null hypothesis (Kass and Raftery 1995).

**Goodness-of-Fit Analyses:** Tests of goodness-of-fit were computed using the following chi-square test criterion:

$$\chi^2 = \sum \frac{(observed - expected)^2}{expected}$$

Null hypotheses were that the data were consistent with the progeny ratios expected under a specific model of inheritance and alternative hypotheses were that the data were inconsistent with the null hypothesis. The degrees of freedom were equal to the number of comparisons minus one (genotypes within hypotheses - 1) (Steel and Torrie 1980, Zar
Bayesian Analyses: Assignment of Prior Probabilities: The focus of this study concerns whether the inheritance patterns of allozyme bands in *A. biternata* are disomic or tetrasomic. There is only one model for tetrasomic inheritance but there may be several hypotheses for disomic inheritance which each correspond to different associations of alleles in the "parental" species (see Figure 4.1). Because there are more disomic than tetrasomic hypotheses, there is a higher probability that a disomic hypothesis will be chosen as correct purely by chance. Therefore, so as not to bias the statistical tests toward disomic inheritance models, prior probabilities were assigned in two ways. First, because my main interest was in determining whether inheritance was disomic or tetrasomic, I assumed the occurrence of the two classes of hypotheses (tetrasomic and disomic) were equal (Tables 4.1-4.6: weighted priors). If more than one disomic hypothesis was included in the analysis, the tetrasomic hypothesis was assigned a prior knowledge of $\frac{1}{2}$ and the remaining $\frac{1}{2}$ was distributed among all disomic hypotheses according to their relative probabilities of occurrence. For instance, in Table 4.1 there are 4.2 ways that plant 100 can have allele 1 and 2 in one subgenome and allele 2 and 3 in the other subgenome (disomic hypothesis 1), but only one way that both copies of allele two can be in the same subgenome (disomic hypothesis 2). In Table 4.1 prior probabilities were assigned as follows: 1) Pr(tetrasomic) = $\frac{1}{2}$, 2) Pr(disomic 1) = $\frac{1}{3}$, and 3) Pr(disomic 2) = $\frac{1}{6}$. Second, for simplicity, I assumed no prior knowledge of hypotheses. In this case prior probabilities were equal for all hypotheses (Tables 4.1-4.6: equal priors). When incorporated into the Bayesian analysis this strategy is termed Bayes' postulate (Lee
1989) and allows the prior probabilities to cancel out of the Bayesian equation.

Other types of prior knowledge which could be incorporated into this analysis include estimates of the proportion of meiotic events with multivalent formation or phylogenetic relationships to other autoploids or allopolyploids. Additionally, because each different cross is essentially a replicate of the segregation of PGI alleles in A. biternata (and each cross is a replicate of the segregation of PGM alleles), the posterior probability for each cross within a locus could have been incorporated as the prior probability for a subsequent cross. This approach was not undertaken in this study.

*Computation Bayesian Posterior probabilities:* The multinomial distribution was used to compute the likelihood (Pr (data|hyp)), or the probabilities of observing the progeny genotypes under each hypothesis:

\[
P(data|H_j) = \frac{n!}{n_1!n_2!n_3!n_4!n_5!n_6!} (P_1(H_j))^{n_1}(P_2(H_j))^{n_2}(P_3(H_j))^{n_3}(P_4(H_j))^{n_4}(P_5(H_j))^{n_5}(P_6(H_j))^{n_6}
\]

where \( n \) is the total number of progeny, \( n_i \) is the observed number of progeny of genotype \( i \), and \( P_i(H_j) \) is the expected proportion of genotype \( i \) under hypothesis \( j \).
Bayes' theorem for discrete distributions was used to compute the posterior probability of each hypothesis:

\[
P(H_i|\text{data}) = \frac{P(\text{data}|H_i) P(H_i)}{\sum_{i=1}^{N} P(\text{data}|H_i) P(H_i)}
\]

where \( H_i \) represents a specific disomic or tetrasomic hypothesis and \( P(H_i) \) is the prior probability of hypothesis \( i \). All conditional probabilities were computed using Mathematica 2.1 (Wolfram 1988).

**Interpretation of Bayesian Analyses:** The Bayesian posterior probability is interpreted as the "confidence or degree of belief" in an individual having a particular underlying segregation ratio (see Alexander et al. 1995). Posterior probabilities are also regarded as the "probability that the hypothesis is true given the data" or the "degree to which the data support one hypothesis over another (Edwards 1992, Kass and Raftery 1995)." For instance, if the posterior probability of hypothesis one is 0.90 and that of hypothesis two is 0.10, hypothesis one is nine times more likely than hypothesis two to be the hypothesis which accords with the data. To interpret the posterior probability a Bayes factor is often computed (Kass and Raftery 1995). The Bayes factor is the likelihood ratio or the posterior odds of a hypothesis computed without prior probabilities (this is numerically equivalent to computation with each hypothesis having the same prior probability, Kass and Raftery 1995). Because discrimination of the type of disomic inheritance is not in itself of interest in this study, the Bayes factors are calculated as
follows:

\[
\text{Bayes Factor for disomic hypothesis} = \frac{pr(data|disomic)}{pr(data|not\ disomic)} = \frac{pr(data|disomic)}{pr(data|tetrasomic)} = \frac{pr(data|H_{d1}) + pr(data|H_{d2}) + \ldots + pr(data|H_{di})}{pr(data|H_{t1}) + pr(data|H_{t2}) + \ldots + pr(data|H_{ti})}
\]

where \(H_{di}\) is the \(i\)th disomic hypothesis, and
\(H_{ai}\) is the \(i\)th tetrasomic hypothesis (there is only one in this case).

A Bayes factor between 3 and 20 is positive evidence supporting the null hypothesis, a Bayes factor greater than 20 is strong evidence in favor of a null hypothesis, and a Bayes factor greater than 150 is very strong evidence supporting the null hypothesis (Kass and Raftery 1995).

The null hypothesis of no linkage between the \(PGI\) and \(PGM\) loci in \(A.\ biternata\) was tested using the Fisher’s Exact test for a 2x4 contingency Table (Weir 1990). The analysis was run using PROC FREQ on SAS for Windows release 6.11 (SAS Institute 1993).

RESULTS

\(\text{Allozyme Banding Patterns at PGI and PGM}\): Initial allozyme screens revealed two loci at the dimeric \(PGI\) locus. The \textit{fast} locus (\(F\)) was monomorphic for all individuals screened. However, the \textit{slow} locus (\(S\)) revealed a total of 4 alleles. Because the \(S\) locus was the only polymorphic \(PGI\) locus and no analysis uses the \(F\) locus, in this
paper PGI-S is referred to as PGI. Dark staining bands were assumed to represent more than one copy of a homodimeric allozyme allele while light homodimeric bands represented only one allele. Some individuals appeared to have 3 alleles at one locus, represented by six bands, three homodimers and three heterodimers.

The monomeric enzyme PGM was not as easy to interpret because the intensity of banding did not always clearly reveal the number of copies of putative alleles at this locus. For this reason only the presence of PGM alleles was scored and not their numbers. Four alleles were identified at the PGM locus surveyed here and some individuals appeared to have 3 alleles at this locus. Because I did not know a priori whether these bands represented one or two loci, I calculated the expected proportions of progeny for several 2 locus-2 allele hypotheses for cross 103x123. After analyzing the posterior probabilities of several possible two locus hypotheses (only the two most likely are shown in Table 4.5), I concluded that the banding was a result of segregation at one locus because no two locus hypothesis explained the data as well as the one locus hypotheses (Bayes Factor = 7.3 x 10²; see Table 4.5).

**Inheritance of Alleles at PGI:** In all cases, patterns of inheritance of alleles at the slow PGI locus are best described by a disomic inheritance model (see Tables 4.1-4.4). Cross 100 x 104 produced all possible genotypes. However, the distribution of progeny genotypes was most likely a result of disomic segregation (Bayes Factor = 31.3; Table 4.1). Over 95% of the posterior probability was assigned to the disomic 1 hypothesis, with the associations of alleles within subgenomes for plant 100 and 104 as listed in Table 4.1.
Only two *PGI* genotypes were found among the progeny of cross 123 x 103 (Table 4.2). These two were the only genotypes predicted by the first disomic hypothesis, which was the most likely (Bayes Factor = $1.2 \times 10^3$; see Table 4.2). Similarly, only two *PGI* genotypes were found among the 120 x 121 progeny and the disomic 1 hypothesis (see Table 4.3) was the most likely explanation for the inheritance pattern (Bayes Factor = $2.1 \times 10^9$). Progeny from plants 105 and 106 were also much more likely to have been produced from parents exhibiting disomic segregation patterns than parents exhibiting tetrasomic segregation (Bayes Factor = 82; Table 4.4).

**Inheritance of Alleles at PGM**: Patterns of inheritance at the *PGM* locus were most likely the result of disomic inheritance, consistent with the allotetraploid origin of the genome. Only four genotypes were found among the progeny of cross 103 x 123 and their segregation ratios were consistent with the disomic 1 hypothesis (Bayes Factor = $6.3\times10^5$; Table 4.5). Patterns of inheritance of *PGM* alleles for progeny of the 100 x 104 cross were also most likely the result of disomic inheritance; however, there was not sufficient data to be strongly confident of the disomic hypothesis (Bayes Factor = 5; Table 4.6).

**Linkage of PGI and PGM**: The null hypothesis of no linkage between *PGI* and *PGM* was tested using the progeny of one cross, 123 x 103. This was the only cross in this study which had variation at both the *PGI* and *PGM* loci and for which I had sufficient sample sizes to test whether *PGI* and *PGM* were linked. Fisher's exact test for a 2 x 4 contingency table did not reject the null hypothesis of no linkage between *PGI* and *PGM* (two-tail probability = 0.42). Therefore, I concluded that *PGM* and *PGI* were not
linked and may represent inheritance patterns of 2 of the 7 homeologous chromosome sets in *Astilbe biternata*.

**DISCUSSION**

**COMPARISON OF ANALYSES:** Overall, the goodness-of-fit and Bayesian analyses gave similar results. However, in a few cases inconsistency between the Bayesian and goodness-of-fit analyses illustrates why caution should be used when multiple hypotheses are being evaluated with chi-square test criteria. First, the $\chi^2$ test incorrectly failed to reject both the tetrasomic and disomic hypotheses when the Bayesian analysis strongly favored the hypothesis of disomic inheritance for *PGI* in the cross 100 x 104 (Table 4.1). One reason for this inconsistency could have been the low sample size. Second, the $\chi^2$ test rejected the hypothesis for tetrasomic inheritance of *PGM* for cross 100x104 whereas the Bayesian analysis only slightly favored the disomic hypothesis (Table 4.6). The conclusion from both analyses is consistent, but the Bayesian analysis urges caution when concluding that the data do not fit the tetrasomic hypothesis. Further sampling should be done before a definitive conclusion can be made. Finally, when multiple independent hypotheses are being tested with chi-square test criteria it is often recommended that the error rate ($\alpha$) be divided by the number of independent tests to avoid inflation of the probability of type I errors. If I had taken this approach, the $\chi^2$ test would not have rejected the following hypotheses: 1) the hypothesis for tetrasomic inheritance in cross 105x106 (Table 4.4), 2) the first two locus/disomic hypothesis for *PGM* cross 123 x 103 presented in Table 4.5, and 3) the hypothesis for tetrasomic inheritance of *PGM* in cross
100 x 104 (Table 4.6). More data would have been necessary to distinguish among hypotheses for these crosses. Although, it is often prudent to be conservative when assessing test statistics, it is inefficient to collect far more data than is needed. The Bayesian analysis appears to be better able to discriminate among multiple hypotheses and determine in which cases it is necessary to collect more data before accepting or rejecting an hypothesis.

The Bayesian analysis used in this study is an appropriate analysis for discriminating among multiple hypotheses. However, because the chi-square analysis provides a "P-value" and the Bayesian analysis does not, some researchers may feel somewhat uncomfortable about how to interpret the Bayesian posterior probabilities. The "P-value" indicates the probability of obtaining a larger $\chi^2$ by chance under the null hypothesis or the probability of rejecting the null hypothesis as a consequence of chance variation in the data when the null hypothesis is true. To estimate a "P-value" for a Bayesian posterior probability, I performed a bootstrapping experiment to compute the probability of obtaining support from the Bayesian analysis for the disomic hypothesis by chance variation in the data when the tetrasomic hypothesis is actually true. The following question was asked: Assuming the tetrasomic hypothesis for the cross in Table 4.1 is true, what is the probability of getting $\Pr(\text{tetrasomic hypothesis} | \text{data}) < 0.046$ (this value was the posterior probability actually calculated for the tetrasomic hypothesis in Table 4.1) ? Using Mathematica 2.1 (Wolfram 1991), I drew a random sample of size 38 with replacement (the same as my sample size from Table 4.1) from the distribution
defined by the tetrasomic hypothesis in Table 4.1, and I computed the Bayesian posterior probability \( \text{Pr}(\text{tetrasomic hypothesis}|\text{data}) \). The tetrasomic hypothesis was assigned a prior probability of \( \frac{1}{2} \), the first and second disomic models were assigned a prior probabilities of \( \frac{1}{3} \) and \( \frac{1}{6} \), respectively. This procedure was repeated 10,000 times. The chance of the posterior probability being less than 0.046, when the null hypothesis of tetrasomic inheritance was true, was 0.0023. This result was in accord with the interpretation of the Bayes Factors from the Bayesian analysis suggesting strong support of the disomic hypothesis.

A likelihood analysis (Edwards 1992) results in the same conclusions as the Bayesian analysis presented herein. However, the Bayesian and likelihood analyses differ in their interpretation and in the manner in which they incorporate prior information. Bayesian analysis is interpreted by assessing the posterior probabilities for each model given the observed data, whereas the significance of the likelihood test must be evaluated assuming a null hypothesis (in this case tetrasomic inheritance). This presents a problem when discriminating among multiple hypotheses using the likelihood test because only two hypotheses can be contrasted at a time. Another difference is that prior information is explicitly incorporated into the Bayesian analysis when computing the posterior probability whereas likelihood analysis incorporates prior knowledge as “prior support” which is added to the log-likelihood ratio. If the prior probabilities of all hypotheses are equal, the Bayesian and likelihood analyses are numerically identical.

The application of the Bayesian analysis presented here is not restricted to
discriminating between disomic and tetrasomic inheritance in tetraploids. On the contrary, this analysis is quite generalizable and can be used whenever discrimination among hypotheses is necessary.

**Inheritance in Astilbe**: All crosses indicated that disomic segregation of alleles occurred at both the *PGI* and *PGM* loci in *Astilbe biternata*. These data support J.L. Hamel's hypothesis (1953) that *A. biternata* is an allotetraploid. Because *PGI* and *PGM* are unlinked, the alleles at these loci are probably located on 2 of the 7 homeologous chromosomes sets in *A. biternata*. More data from segregation patterns at other unlinked loci are needed to determine whether disomic inheritance occurs for all chromosome sets, which if true would lend further credence to the hypothesis of the allopolyploid origin of *A. biternata*. Caution should be exercised, however, when concluding an allotetraploid origin from disomic inheritance of genetic markers. Although tetrasomic inheritance appears to be sufficient to conclude autopolyploid speciation, disomic inheritance is not sufficient to conclude allopolyploid speciation because a species with disomic inheritance may have originated via autopolyploid speciation and subsequently evolved disomic inheritance patterns for all chromosomes (Sybenga 1969). Further evidence for allopolyploid origins can be gathered by looking for the absence of multivalent formation during meiosis and attempting to reconstruct hybrid allopolyploids from their putative diploid progenitors (Grant 1981).

When the genome from each parental species is homozygous for a different allele the allotetraploid hybrid will exhibit “fixed heterozygosity.” In other words, each gamete
from the tetraploid will contain one allele from each parental genome, and because alleles are different, all gametes will be heterozygous. “Fixed heterozygosity” is a sufficient condition to describe the disomic inheritance patterns which characterize allotetraploids; however, the patterns of associations of PGI and PGM alleles in this study show that it is not necessary. Of the subgenomes studied here, only 3 of 8 PGI subgenomes (one on each of plants 120, 121, and 123) and one of the 4 PGM subgenomes (123) exhibited “fixed heterozygosity”. These results suggest that it is necessary to investigate inheritance patterns in tetraploids beyond the acknowledgment of “fixed heterozygosity” (for other examples of disomic inheritance see Arulsekar et al. 1981, Hart 1983, Quiros and McHale 1985, Watson et al. 1991, Beaver and Iezzoni 1993).

**Implications for polyploid speciation in the Saxifragaceae:** This study documents the presence of disomic inheritance and a putative allotetraploid in the Saxifragaceae. It is interesting to note that within the Saxifragaceae, Astilbe+Saxifragopsis is in the clade with the Boykinia group+Leptarrhena+Tanakaea and does not appear to be closely associated with the Heuchera group where tetrasomic inheritance and autopolyploidy have been found repeatedly (Ness et al. 1989, Soltis 1984, Wolf et al. 1990). The hypothesis that autotetraploidy may be more common in some plant groups than others may not be true at the family level in the Saxifragaceae, but it may be supported at a lower taxonomic level. The presence of multiple origins of autotetraploidy and allotetraploidy in the Saxifragaceae suggest that it may be an interesting family in which to study taxonomic associations of autopolyploidy and
allopolyplody. Future studies may be able to address whether certain lineages are
predisposed to speciate via autopolyplody rather than allopolyplody and why this may
be the case.

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Bill Morris, Peter Tiffin, and an anonymous reviewer. Thanks to Chris Richards and Paul
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Improvement Grant from the National Science Foundation (DEB-9520754) on behalf of
Matt Olson to Janis Antonovics and Jim Clark.
Table 4.1. Analysis of segregation ratios at the PGI locus for cross 100 (1223) x 104 (2333)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>Hypotheses</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tetrasomic</td>
<td>disomic 104 (23)(33)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/4</td>
</tr>
<tr>
<td>2233</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>1233</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>1223</td>
<td>1/6</td>
<td>1/8</td>
</tr>
<tr>
<td>2333</td>
<td>1/6</td>
<td>1/8</td>
</tr>
<tr>
<td>1333</td>
<td>1/12</td>
<td>1/8</td>
</tr>
<tr>
<td>2223</td>
<td>1/12</td>
<td>1/8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(\chi^2)</th>
<th>Ln ((\mathcal{L}))</th>
<th>Pr(hypldata)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.6 (ns)</td>
<td>-13.86</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.0 (ns)</td>
<td>-10.44</td>
<td>-\infty</td>
</tr>
<tr>
<td>weighted priors</td>
<td>0.061</td>
<td>0.939†</td>
<td>0.0</td>
</tr>
<tr>
<td>equal priors</td>
<td>0.031</td>
<td>0.969†</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given \(\frac{1}{2}\) of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = P >0.05, * = P<0.05, ** = P<0.01. For Bayesian analyses: \(\dagger\) = Strong support, \(\dagger\dagger\) = Very Strong support
Table 4.2. Analysis of segregation ratios at the PGI locus for cross 123 (2233) x 103 (2333)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>Hypotheses</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tetrasomic</td>
<td>disomic</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>123 (22)(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>103 (23)(33)</td>
</tr>
<tr>
<td>2223</td>
<td>1/12</td>
<td>0</td>
</tr>
<tr>
<td>2233</td>
<td>5/12</td>
<td>½</td>
</tr>
<tr>
<td>2333</td>
<td>5/12</td>
<td>½</td>
</tr>
<tr>
<td>3333</td>
<td>1/12</td>
<td>0</td>
</tr>
</tbody>
</table>

$\chi^2$ 38.35 ** 0.64 (ns) 50.38**

Ln (\$\ell\$) -9.49 -2.38 -13.60

Bayes Pr (hypldata)

<table>
<thead>
<tr>
<th></th>
<th>weighted priors</th>
<th>equal priors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00163</td>
<td>0.9998396</td>
</tr>
<tr>
<td></td>
<td>2.76x10^-10</td>
<td>1.34x10^-5</td>
</tr>
</tbody>
</table>

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given ½ of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = P >0.05, * = P<0.05, ** = P<0.01. For Bayesian analyses: †† = Strong support, ††† = Very Strong support.
Table 4.3. Analysis of segregation ratios at the PGI locus for cross 120 (2234) x 121 (2233)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>tetrasomic</th>
<th>disomic 120 (22)(34)</th>
<th>disomic 120 (23)(44)</th>
<th>disomic 120 (22)(34)</th>
<th>disomic 120 (23)(24)</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>2222</td>
<td>1/36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/16</td>
<td>0</td>
</tr>
<tr>
<td>2223</td>
<td>6/36</td>
<td>0</td>
<td>1/4</td>
<td>1/8</td>
<td>3/16</td>
<td>0</td>
</tr>
<tr>
<td>2233</td>
<td>9/36</td>
<td>½</td>
<td>1/4</td>
<td>1/4</td>
<td>3/16</td>
<td>14</td>
</tr>
<tr>
<td>2333</td>
<td>2/36</td>
<td>0</td>
<td>0</td>
<td>1/8</td>
<td>1/16</td>
<td>0</td>
</tr>
<tr>
<td>2224</td>
<td>2/36</td>
<td>0</td>
<td>0</td>
<td>1/8</td>
<td>1/16</td>
<td>0</td>
</tr>
<tr>
<td>2234</td>
<td>9/36</td>
<td>½</td>
<td>1/4</td>
<td>1/4</td>
<td>3/16</td>
<td>17</td>
</tr>
<tr>
<td>2334</td>
<td>6/36</td>
<td>0</td>
<td>1/4</td>
<td>1/8</td>
<td>3/16</td>
<td>0</td>
</tr>
<tr>
<td>3334</td>
<td>1/36</td>
<td>0</td>
<td>0</td>
<td>1/8</td>
<td>1/16</td>
<td>0</td>
</tr>
</tbody>
</table>

χ²    31.58 **     0.29 (ns)    31.58 **    31.58 **    52.44 **  
\[\ln(\mathcal{L})\] -23.58    -2.09    -23.58    -23.58    -32.50  
\[\text{Pr(hyp|data)}\] weighted priors \(1.86 \times 10^{-9}\) \(\approx 1. \dagger\dagger\) \(4.7 \times 10^{-10}\) \(4.7 \times 10^{-10}\) \(6.2 \times 10^{-14}\)  
equal priors \(4.6 \times 10^{-10}\) \(\approx 1. \dagger\dagger\) \(4.6 \times 10^{-10}\) \(4.6 \times 10^{-10}\) \(6.2 \times 10^{-14}\)

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given \(1/2\) of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = \(P > 0.05\), * = \(P < 0.05\), ** = \(P < 0.01\). For Bayesian analyses: \(\dagger\) = Strong support, \(\dagger\dagger\) = Very Strong support.
Table 4.4. Analysis of segregation ratios at the PGI locus for cross 106 (2233) x 105 (1222)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>Hypotheses</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tetrasomic</td>
<td>disomic 106 (22)(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105 (12)(22)</td>
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<tr>
<td>1222</td>
<td>1/12</td>
<td>0</td>
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<tr>
<td>1223</td>
<td>4/12</td>
<td>½</td>
</tr>
<tr>
<td>1233</td>
<td>1/12</td>
<td>0</td>
</tr>
<tr>
<td>2222</td>
<td>1/12</td>
<td>0</td>
</tr>
<tr>
<td>2223</td>
<td>4/12</td>
<td>½</td>
</tr>
<tr>
<td>2233</td>
<td>1/12</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ \chi^2 \quad 13.6 \ast \quad - \quad 3.23 \text{ (ns)} \]

\[ \text{Ln(}\Phi) \quad -17.50 \quad -\infty \quad -13.06 \]

\[ \text{Pr(hypo\text{data})} \quad \text{weighted priors} \quad 0.023 \quad 0.0 \quad 0.977 \dagger \]

\[ \text{equal priors} \quad 0.012 \quad 0.0 \quad 0.988 \dagger \]

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given ½ of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = P >0.05, \ast = P<0.05, ** = P<0.01. For Bayesian analyses: \dagger = Strong support, \dagger \dagger = Very Strong support
Table 4.5. Analysis of segregation ratios at the PGM locus for cross 123 (2245) x 103 (2234)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>Hypotheses</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tetrasomic</td>
<td>disomic</td>
</tr>
<tr>
<td></td>
<td>123 (22)(45)</td>
<td>123 (22)(45)</td>
</tr>
<tr>
<td></td>
<td>103 (22)(34)</td>
<td>103 (23)(24)</td>
</tr>
<tr>
<td>2</td>
<td>1/36</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>2/36</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>8/36</td>
<td>1/4</td>
</tr>
<tr>
<td>25</td>
<td>2/36</td>
<td>0</td>
</tr>
<tr>
<td>234</td>
<td>9/36</td>
<td>1/4</td>
</tr>
<tr>
<td>235</td>
<td>4/36</td>
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<td>7/36</td>
<td>1/4</td>
</tr>
<tr>
<td>345</td>
<td>1/36</td>
<td>0</td>
</tr>
<tr>
<td>2345</td>
<td>4/36</td>
<td>0</td>
</tr>
</tbody>
</table>

|                  |            |                  |                  |                  |            |                  |                  |
|                  | $\chi^2$  | 29.18 **         | 2.74 (ns)        | 23.77 **         | 28.38 **    | 62.88 **         | 16.66 *          | 23.77 **         |
|                  | Ln(δf)    | -20.22           | -6.87            | -20.08           | -22.12      | -32.37           | -18.09           | -20.08           |
|                  | Pr(hypdata) |                  |                  |                  |            |                  |                  |
| weighted priors  |            | 1.0x10^{-6}      | 0.999983††       | 1.9x10^{-6}     | 2.4x10^{-7} | 8.4x10^{-12}     | 1.3x10^{-5}      |                           |
| equal priors     |            | 1.7x10^{-7}      | 0.999985††       | 1.9x10^{-6}     | 2.4x10^{-7} | 8.4x10^{-12}     | 1.3x10^{-5}      | 1.9x10^{-6}      |

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given $\frac{1}{2}$ of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = P > 0.05, * = P < 0.05, ** = P < 0.01. For Bayesian analyses: † = Strong support, †† = Very Strong support
Table 4.6. Analysis of segregation ratios at the PGM locus for cross 104 (2234) x 100 (2223)

<table>
<thead>
<tr>
<th>Progeny Genotype</th>
<th>Hypothesis</th>
<th>Observed Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tetrasomic</td>
<td>disomic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>104 (23)(24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 (22)(23)</td>
</tr>
<tr>
<td>2</td>
<td>1/12</td>
<td>1/8</td>
</tr>
<tr>
<td>23</td>
<td>5/12</td>
<td>3/8</td>
</tr>
<tr>
<td>24</td>
<td>2/12</td>
<td>1/8</td>
</tr>
<tr>
<td>234</td>
<td>4/12</td>
<td>3/8</td>
</tr>
</tbody>
</table>

\[ \chi^2 \] = 8.09 *  
\[ \text{Ln}(\mathcal{L}) \] = -8.29  
\[ \text{Pr(hypldata)} \] equal priors = 0.164  
\[ \text{Pr(hypldata)} \] weighted priors = 0.282

Each column under hypotheses gives the proportions of each genotype expected under the indicated hypothesis. For disomic hypotheses the association of alleles in the subgenomes are indicated within parentheses. Bayesian Posterior Probabilities of the hypotheses given the data were calculated in two ways: 1) equal priors: the prior probabilities of all hypotheses were equal and 2) weighted priors: the tetrasomic hypothesis was given ½ of the prior probability and the other half was evenly divided among the disomic hypotheses. For chi-square analyses: (ns) = P >0.05, * = P<0.05, ** = P<0.01. For Bayesian analyses: † = Strong support, †† = Very Strong support.
Figure 4.1. Simplified diagram of all possible associations of alleles and their inheritance for the disomic 1 and disomic 2 hypotheses in Table 4.1. Markers on duplicated chromosomes (during metaphase I) are identified by a number and a subscript denoting the subgenome with which the allele is associated (e.g. I_A = allele I subgenome A). Each subgenome contributes one allele to the diploid gamete. Under the disomic 1 hypothesis there are two possible alignments of chromosomes along the metaphase plate for plant 100 (1234), and four types of gametes are produced in equal frequencies. Plant 104 produces only two types of gametes. Note that recombination among homeologous chromosomes does not change the types of gametes produced, only the relative ratios in which they are produced. The probability of any particular progeny genotype is computed as the product of the frequencies of gametes which result in that genotype.
Example calculations of progeny frequencies

Pr (2233) = 0.5 (2, 3 from 104) * 0.25 (2, 3 from 100) + 
0.5 (3, 3 from 104) * 0.25 (2, 2 from 100) = 0.25
Pr (1223) = 0.5 (2, 3 from 104) * 0.25 (1, 2 from 100) = 0.125
Pr (1333) = 0
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Biography

MATTHEW S. OLSON

Department of Botany
P.O. Box 90339
Duke University
Durham, NC 27708-0339
E-mail: ols@acpub.duke.edu
phone: (919) 684-3715

EDUCATION

DUKE UNIVERSITY, 1992-Present
Ph.D. Candidate, Botany,
Advisor: Dr. Janis Antonivics
"The Genetics and Evolution of Subdioecy in Astilbe biternata"

LOUISIANA STATE UNIVERSITY, 1989-1992
Master of Science in Botany / Minor in Experimental Statistics,
Advisor Dr. William Platt
"Effects of Early and Late Growing Season Fires on Resprouting of Shrubs in Upland
Longleaf Pine Savannas and Embedded Seepage Savannas"

ORGANIZATION FOR TROPICAL STUDIES, Spring 1991
Tropical Biology an Ecological Approach

UNIVERSITY OF TEXAS AT AUSTIN, 1984-1987
Bachelor of Arts in Biology / Minor in Chemistry

TEACHING EXPERIENCE AND AWARDS

DUKE UNIVERSITY
Fellow at the Center for Teaching and Learning at Duke University 1996-1997
Teaching Assistant for Principles of Evolution, Ecology and Society, Field Botany,
Genetics and Cell Biology, and General Biology from Spring 1993 to present

LOUISIANA STATE UNIVERSITY
Teaching assistant for General Botany and General Biology from 1989 to 1992

HONORS AND GRANTS

DUKE UNIVERSITY
NSF and Sloan Postdoctoral reseach Fellowship in Molecular Evolution 1998-1999
NSF Doctoral Dissertation Improvement Award, September 1995-September 1997
"The Genetics and Evolution of Subdioecy in Astilbe biternata"
Alcane Webb Dissertation Research Award, 1995
Sigma Xi Grant-in-Aid, 1994, 1995
Katherine Keever Award, 1993, 1994
Teaching Assistantship 1992-1996
Graduate Travel Award 1995, 1996
Louisiana State University
Honorable Mention NSF Graduate Fellowship Awards, 1990
Sigma Xi Grant-in-Aid, 1990
Teaching Assistantship, 1989-1992
University of Texas at Austin
Dean's Scholar, 1984-1987

Publications


Abstracts and Presentations

1996 Botanical Society of America Meetings; Contributed Paper
“Application of maximum likelihood tests to discriminate between tetrasomic and disomic inheritance in the tetraploid A. biternata (Saxifragaceae)”

1995 Ecological Society of America Meetings; Poster
“Reproductive success through ovules of females and hermaphrodites in the subdioecious herb, A. biternata”

1993 Ecological Society of America Meetings; Contributed Paper
“Recovery of shrubs in longleaf pine forests and embedded seepage savannas following early and late growing season burns”

1991 Tall Timbers Ecology Conference; Poster
“Recovery of shrubs in longleaf pine forests and embedded seepage savannas following early and late growing season burns”

Professional Affiliations

Ecological Society of America, Organization for Tropical Studies, Sigma Xi