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The effects of light intensity on the flowering phenology and inter-population differences of the androdioecious plant species, *Mercurialis annua*

Andrea Benedict

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Abstract

Mercurialis annua is an androdioecious plant species, meaning populations may contain both male and cosexual individuals. It is able to modify resource allocation to male and female function in varying conditions, such as light and competition regimes. One component of resource allocation may involve changes in the relative timing of production of male and female flowers. A controlled greenhouse experiment was performed to test for the effects of shading on the production of male and female flowers in four populations. Evidence suggested that plants grown in shade would be relatively less female than those grown in the sun. Although the average number of day to the production of male versus female flowers did not show a response to shading, the number of plants producing male versus female flowers during early development differed between light treatments. The shade treatment induced a reduction in number of plants producing female flowers, as predicted by my hypothesis. The populations differed in their specific responses to shading, suggesting that populations have adapted individually to local selective pressures.

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Dr. Gary Hannan

Second Advisor

Dr. James Vandenbosch

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THE EFFECTS OF LIGHT INTENSITY ON THE FLOWERING PHENOLOGY AND INTER-POPULATION DIFFERENCES OF THE ANDRODIOECIOUS PLANT SPECIES, *MERCURIALIS ANNUA*

by

Andrea Benedict

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ABSTRACT

Author: Andrea L. Benedict
Department: Biology
Supervising Instructor: Dr. Gary Hannan
Honors Advisor: Dr. James Vandenbosch
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Mercurialis annua is an androdioecious plant species, meaning populations may contain both male and cosexual individuals. It is able to modify resource allocation to male and female function in varying conditions, such as light and competition regimes. One component of resource allocation may involve changes in the relative timing of production of male and female flowers. A controlled greenhouse experiment was performed to test for the effects of shading on the production of male and female flowers in four populations. Evidence suggested that plants grown in shade would be relatively less female than those grown in the sun. Although the average number of day to the production of male versus female flowers did not show a response to shading, the number of plants producing male versus female flowers during early development differed between light treatments. The shade treatment induced a reduction in number of plants producing female flowers, as predicted by my hypothesis. The populations differed in their specific responses to shading, suggesting that populations have adapted individually to local selective pressures.

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Introduction:

Mercurialis annua is a wind-pollinated, self-compatible, functionally androdioecious plant species (Pannell 1997b). Androdioecy is a breeding system in which there are both male and monoecious (cosexual) individuals coexisting in the same population (Pannell 1997b). Androdioecy is a very rare breeding system and little is known about the mechanisms by which males are maintained in a population (Barrett 2002).

All plants must partition the resources obtained from their environment between growth and reproductive function (Obeso 2002). Cosexual plants, having both male and female structures, must then partition the resources made available for reproduction in a way that maximizes individual fitness (Parachnowitsch and Elle 2004). This partitioning to maximize individual fitness is a key concept in sexual allocation theory (Parachnowitsch and Elle 2004).

Female reproductive structures and the fruits they produce are more energetically costly then male reproductive structures (Parachnowitsch and Elle 2004). The plants also must dynamically allocate resources for reproduction at sequential nodes as the plants increase in size (Kaitaniemi and Honkanen 1996). *Mercurialis annua* is unusual in being androdioecious, where about 70% of plants in most populations are cosexual and about 30% are genetically male (Pannell, 1997a). Thus the sexual allocation theory may be applied to the cosexual individuals in the population, but not the male plants, which are only allocating resources to male reproductive function. Little is known about exactly how androdioecy arose in this plant species, or why it may be advantageous to maintain 30% of the population as genetically male plants (Pannell 1997a). Evolutionary pressures may cause cosexual plants to modify their allocation of resources to male versus female reproductive function (Obeso 2002).

One very curious feature about *Mercurialis annua* is that, although maleness is genetically determined by a single gene locus, some environmental factors, such as a decrease in local competition, can cause some genetic males to function as cosexuals later in their developmental process, and an increase in local competition can produce cosexes with female-biased sex allocation (Pannell 1997c). Populations of *Mercurialis annua* in the wild show great genetic reproductive diversity by being present in stands populations of completely dioecious diploid individuals, and whereas other populations in other stands, which are hexaploid and androdioecious (Pannell 1997b).

Another interesting attribute of this species is that the cosexual plants display protogyny, which is the development of female reproductive organs before male organs, a flowering behavior that to avoidreduces self-fertilization (Pannell 1997b). In a field study, Pannell (1997a) found that shading by trees directly affected allocation for reproduction; male plants reduced their allocation for pollen production, whereas cosexuals reduced their allocation for female function, making the cosexuals quantitatively more male. Since this was a field study it was not known whether some other factor might be contributing to the shift towards maleness in cosexuals (Pannell 1997a).

An additional aspect of sexual reproduction in these plants may be in the timing of the onset of female verses male reproduction. Previous work on sex allocation showeds that *Mercurialis annua* is phenotypically plastic with respect to sexual function (Pannell, 1997a,b,c). The objective of this experiment was to test the effects of light intensity on flowering phenology in a greenhouse setting, while experimentally controlling other factors that might influence flowering phenology.

My hypothesis is that if a reduction in female function occurs in shaded conditions, then plants will either delay the onset of female flowering or fewer plants will produce female flowers during the early stages of floral developmentfor which the plants were observed. In addition, if populations are genetically variation in regardsvariable with respect to control of their sexual function, then response to shading will vary between populations.

Methods:

Mercurialis annua seeds were collected by Dr. John Pannell from two southern Portugal populations (populations A and B) and two southern Spain populations (populations C and D). A total of two hundred sixty-four264 2.5-inch pots were were used. Sixty-six of the pots were used per population, initially with four seeds per pot, planted in Fafard Superfine Germinating Mix™ , on July 22, 2004. Thirty-three pots for each population were randomly placed in either the sun or shade treatments. A total of hundred thirty-two132 pots were placed in three randomly arranged sun treatment blocks and one hundred thirty-two132 pots were placed in three randomly arranged shade tents. Shade tents were constructed of PVC piping with two layers of burlap covering the top and all four sides of the tent. The light level in the greenhouse on December 1, 2004 at 3:10 pm was measured at 125 µmol s^{-1} m^{-2}, and the light level measured within the shade tent was 38 µmol s^{-1} m^{-2}, thus the tents provided 69.6% shade cover. After the plants had germinated, nine days after planting, the pots were thinned down to one plant per pot, with selection for plants with the tallest, darkest green and most robust leaves. Once flowering commenced, the plants were checked regularly, about every two days. Nodes 0 through 5 of the plants were the only nodes observed in this experiment. The date of first flowering and the sex of that flower at each node and at each position (axillary, stalk, and axillary growth) at that node were recorded for each individual plant for each day data was collectedof data collection (Figure 1).

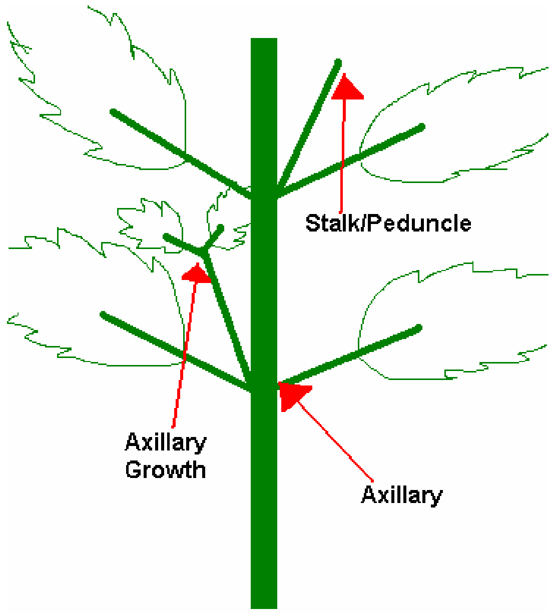

Figure 1. Flowers of both sexes occurred at all three positions, and every node on the plant had the possibility of producing all three positions at one time.

If the opposite sex came up at the same or at a later date at the same node and position where a flower had already openedand/or already been recorded, the date was also recorded for the opening of the opposite sex. Data collection for each of the light treatments ended when more than half of the plants had flowered at the axillary position at each of the first five nodes. The experiment was terminated on October 25, 2004. DThe data analyses were performed on the mean number of days to first flowering using a three-way analysis of variance (ANOVA) statistical test with light, node and sex as factors using SPSS 11 for Macintosh. Since the number of plants producing flowers of either sex was extremely variable and therefore, the ANOVA employed an unbalanced design. Consequently, it was important to look at the number of plants producing flowers of each sexual state. Pairwise , and aChi-Square tests were performed on the number of plants producing flowers at each node in the sun and shade treatments to test for independence between light, node, and sex for the number of plants producing flowers of each sex..

Results:

Axillary Position: Number of Days to Flowering

The three-way ANOVA was used to test for significant effect of light, node, and sex treatments and interactions among them. For all populations, the number of days to first flowering differed between nodes ($p<0.05$) (Table 1). Most of the populations, except population C, showed that the number of days to first flowering differed between light treatments ($p<0.05$) (Table 1). There was no light by sex interaction for any of the populations for the axillary position, which means that light treatment and sex determination are independent of each other when determining the date of first flowering ($p>0.05$). Populations A and C are showingshow more different combinations of significant factors than the other populations and to each other. differences for which factors are significant, as compared to pPopulations B and D, which are showng the same factors as being significant (Table 1).

Table 1. Summary of ANOVA results testing for effects of light, node and sex on number of days to first flowering.

				Interaction		
Population	Light	Node	Sex	Light X Node	Node X Sex	Light X Sex
A	p=0.008	p=0.006	p=0.009	p=0.005	p=0.003	p=0.666
B	p=0.034	p=0.013	p=0.148	p=0.804	p=0.252	p=0.732

C	p=0.083	p=0.001	p=0.183	p=0.577	p=0.495	p=0.080
D	p=0.026	p=0.004	p=0.144	p=0.308	p=0.505	p=0.813

Plants in the sun treatment always started producing flowers earlier than plants in the shade treatment for all populations (Figure 2).

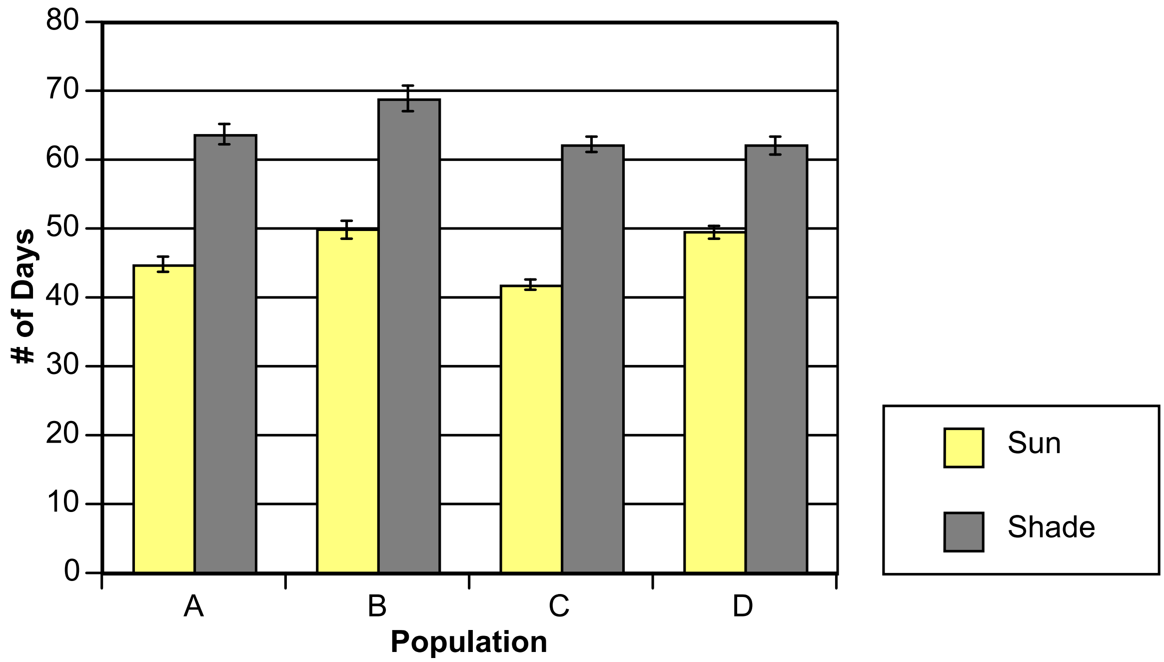

Figure 2. The mean number of days to first flowering is always shorter for the sun treatment for all populations, as compared to the shade treatment.for sun and shade treatments in the four populations. Bars represent $\pm$ 1 standard error.

The node interaction effect can be attributed to the fact that flowers appeared sequentially, starting with node one, for all of the populations (Figures 3-6). All populations show female flowers occurring earlier than male flower at every node where both sexes are present, except population D, node two (Figures 3-6). The ANOVA results for populations B-D show no significant sex effect, but at most nodes female flowers appear earlier than male flowers (Figures 4-6). Population A was the only population to have a significant sex effect in the three-way ANOVA, meaning the day that the first female flower matured was significantly earlier than the first maturing male flower. Population A is the only population that showed both a 'light by node' interaction and a 'node by sex' interaction (Table 1). The 'light by node' interaction shows that light treatment and node number influence each other with regard to the date of first flowering. The 'node by sex' interaction shows that the node number and sex of the flower influence each other in regards to the date of first flowering, which showed that at node two, the female and male flowers appeared at about the same number of days (Figure 3)

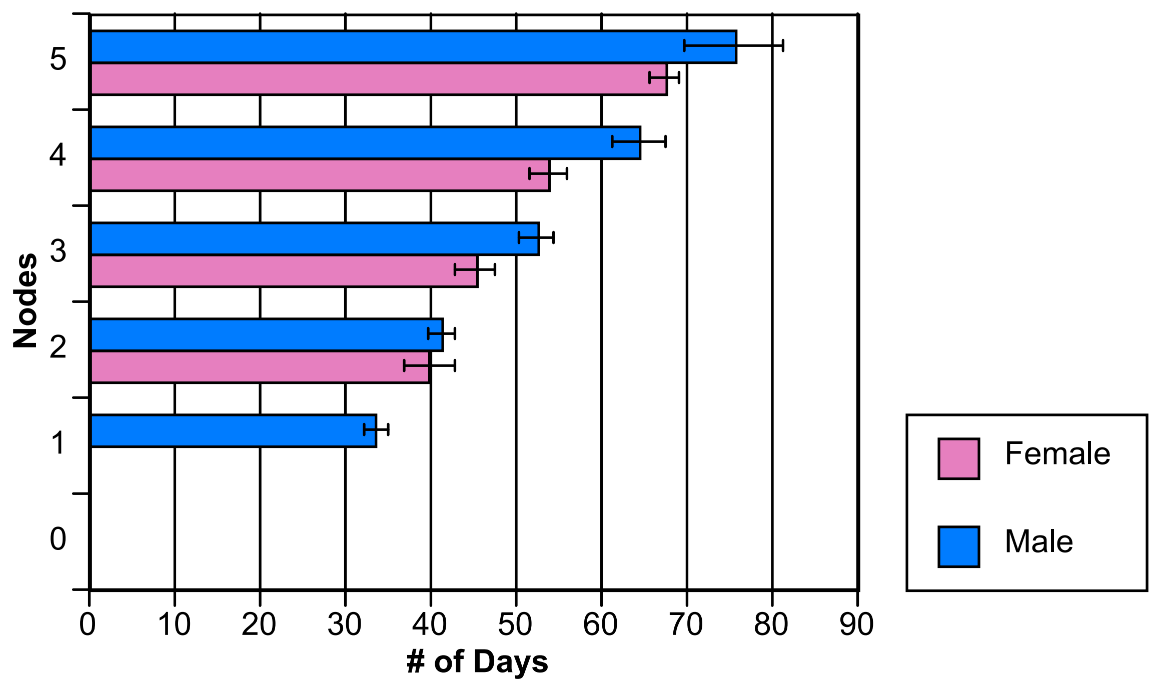

Figure 3. Number of days to maturation of the first flower of each sex at each node for population A. Bars represent ± 1 standard error.

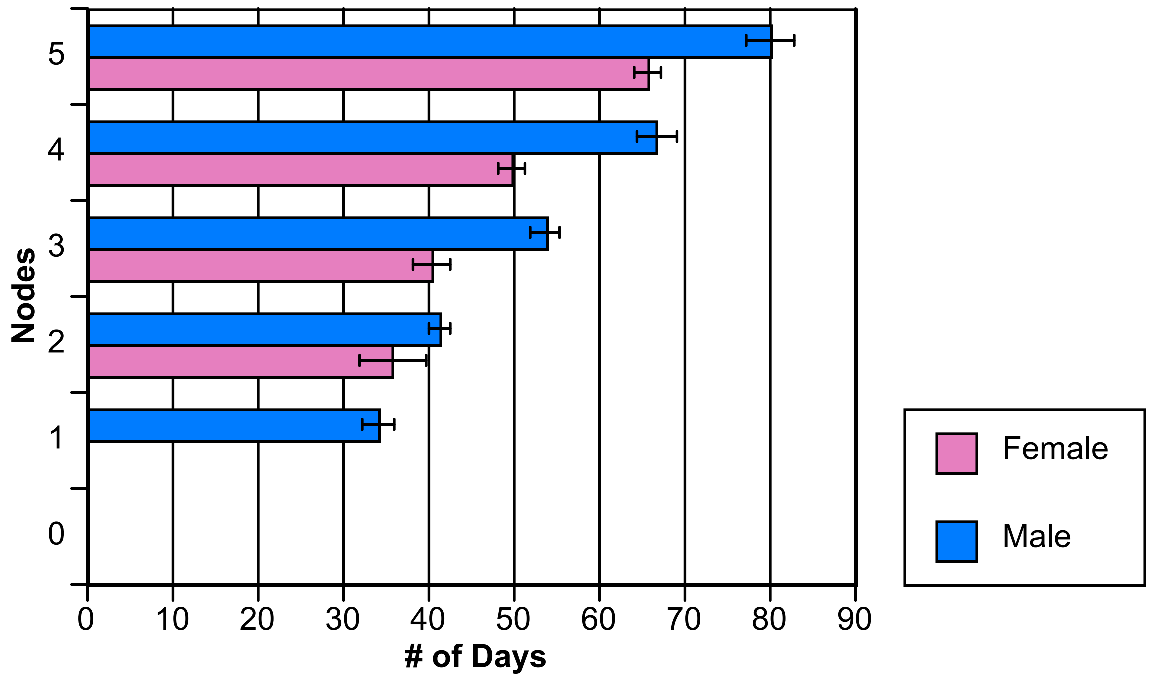

Figure 4. Number of days to maturation of the first flower of each sex at each node for population B. Bars represent ± 1 standard error.

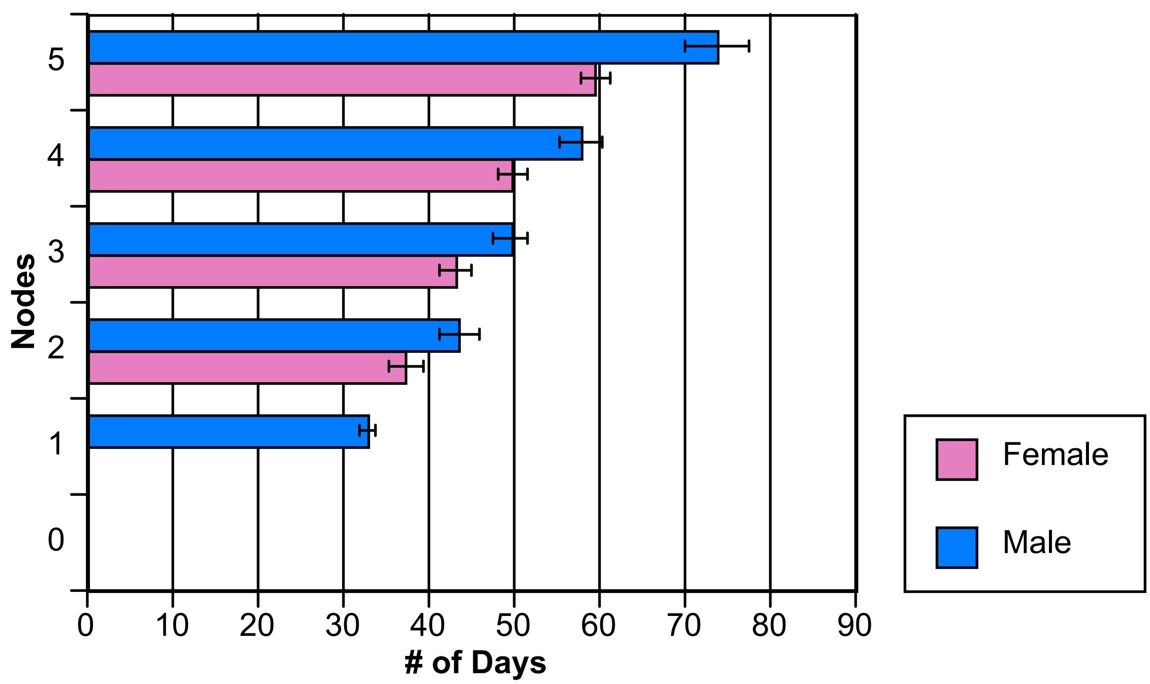

Figure 5. Number of days to maturation of the first flower of each sex at each node for population C. Bars represent ± 1 standard error.

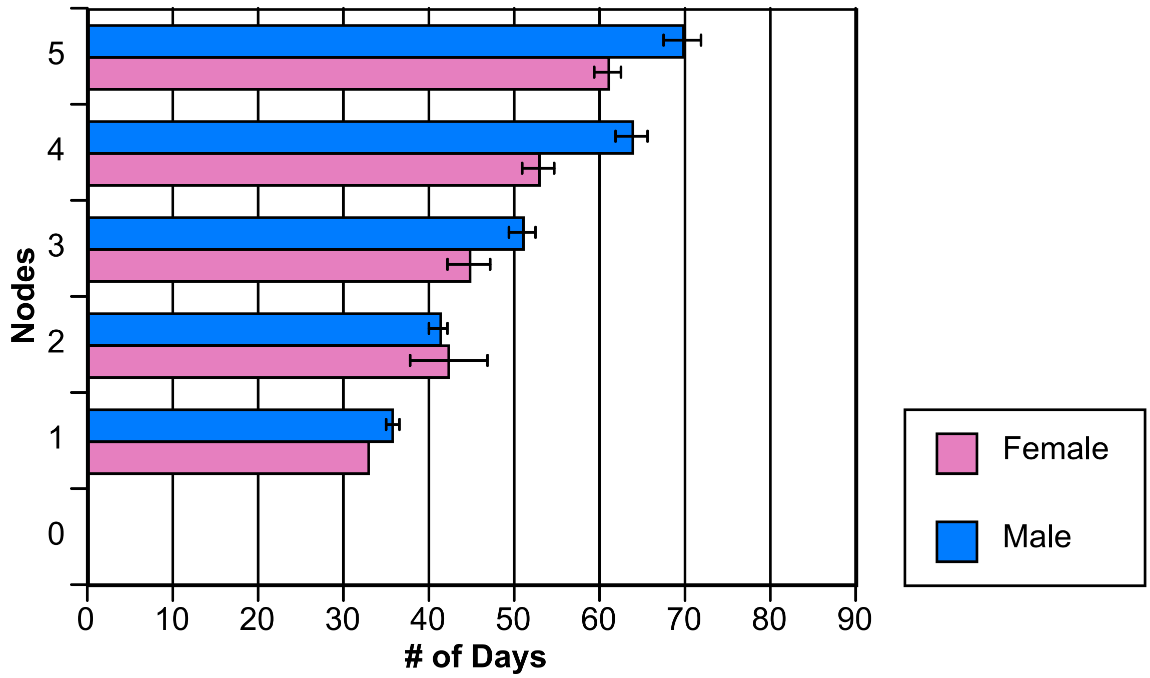

Figure 6. Number of days to maturation of the first flower of each sex at each node for population D. Bars represent ± 1 standard error.

Axillary Position: Number of Plants Producing Flowers

Chi-Square tests were performed on the number of plants producing flowers to look for significant interactions between light and sex, node and light, and node and sex. Populations A and B showed a 'light by sex' interaction, but populations C and D did not (Tables 2-5). Population A had more plants producing male flowers and fewer plants producing male flowers than was expected in the sun treatment, and in the shade treatment there were more plants producing female flowers than expected and fewer plants producing male flowers (Table 2). Population B showed the opposite deviation from expected numbers for the sun and shade treatments (Table 3). The sun treatment had more plants producing female flowers than was expected and fewer plants producing male flowers than was expected, and in the shade more plants produced male flowers and fewer plants produced female flowers than was expected (Table 3). This interaction occurreds when pooling all of the nodes togetherfrom each plant.

Table 2. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population A.

		Sex	
Light		Female	Male
Sun	Observed	63.0	60.0
	Expected	71.4	51.6
Shade	Observed	60.0	29.0
	Expected	51.6	37.4

X^2	5.561
p-value	0.018

Table 3. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population B.

		Sex	
Light		Female	Male
Sun	Observed	50.0	54.0
	Expected	42.0	62.0
Shade	Observed	13.0	39.0
	Expected	21.0	31.0

X^2	7.668
p-value	0.006

Table 4. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population C.

		Sex	
Light		Female	Male
Sun	Observed	89	80
	Expected	94.5	74.5
Shade	Observed	71	46
	Expected	65.5	51.5

X^2	1.805
p-value	0.179

Table 5. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population D.

		Sex	
Light		Female	Male
Sun	Observed	66	89
	Expected	68.8	86.3
Shade	Observed	44	49
	Expected	41.3	51.8

X^2	0.527
p-value	0.468

Populations A and C showed a significant node by light interaction for the number of plants producing flowers, but in populations B and D this interaction was not significant (Tables 6-9). All four populations show the same pattern in the sun by having more plants producing flowers than expected at the lower nodes and then having fewer plants than expected flowering at the upper nodes. The shade treatment showed the opposite effect with fewer plants producing flowers than were expected at the lower nodes and more plants than expected producing flowers at the upper nodes. While this general pattern was seen for all of the populations, only populations A and C deviated enough from the expected numbers to provide significant Chi-Square values.

Table 6. Chi-Square test for independence between node and light treatment with respect to number of plants producing flowers for population A.

		Light	
Node		Sun	Shade
5	Observed	24.0	25.0
	Expected	28.4	20.6
4	Observed	29.0	23.0
	Expected	30.2	21.8
3	Observed	31.0	29.0
	Expected	34.8	25.2
2	Observed	27.0	12.0
	Expected	22.6	16.4
1	Observed	12.0	0.0
	Expected	7.0	5.0
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	13.442
p-value	0.009

Table 7. Chi-Square test for independence between node and light treatment with respect to number of plants producing flowers for population B.

		Light	
Node		Sun	Shade
5	Observed	22.0	16.0
	Expected	25.3	12.7
4	Observed	31.0	18.0
	Expected	32.7	16.3
3	Observed	26.0	14.0
	Expected	26.7	13.3
2	Observed	20.0	4.0
	Expected	16.0	8.0
1	Observed	5.0	0.0
	Expected	3.3	1.7
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	7.121
p-value	0.130

Table 8. Chi-Square test for independence between node and light treatment with respect to number of plants producing flowers for population C.

Node		Light: Sun	Light: Shade
5	Observed	34.0	29.0
	Expected	37.2	25.8
4	Observed	41.0	33.0
	Expected	43.7	30.3
3	Observed	42.0	33.0
	Expected	44.3	30.7
2	Observed	38.0	22.0
	Expected	35.5	24.5
1	Observed	14.0	0.0
	Expected	8.3	5.7
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	11.535
p-value	0.021

Table 9. Chi-Square test for independence between node and light treatment with respect to number of plants producing flowers for population D.

Node		Light: Sun	Light: Shade
5	Observed	38.0	25.0
	Expected	39.4	23.6
4	Observed	39.0	35.0
	Expected	46.3	27.8
3	Observed	39.0	21.0
	Expected	37.5	22.5
2	Observed	33.0	12.0
	Expected	28.1	16.9
1	Observed	6.0	0.0
	Expected	3.8	2.3
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	9.172
p-value	0.057

The 'node by light' interaction was further investigated by analyzing sexes separately for each light treatment. All of the populations showed a 'node by sex' interaction with the sun treatment for the number of plants producing flowers of each sex, but this interaction was not significant in the shade treatment for any of the populations (Tables 10-13). In the sun treatment the number of plants producing female flowers started off fewer plants than the expected number at the lower nodes, and becoming more than expected at the upper nodes. Whereas the number of plants producing male flowers showed the opposite effect. The lower nodes had more plants producing male flowers than expected, and at the upper nodes fewer plants than expected were producing male flowers. This deviation from expected values was not seen in the shade treatment.

Table 10. Chi-Square test for independence between node and sex of flower with respect to number of plants producing flowers of each sex in the sun and shade treatment for population A.

Sun

		Sex	
Node		Female	Male
5	Observed	22.0	2.0
	Expected	12.3	11.7
4	Observed	20.0	9.0
	Expected	14.9	14.1
3	Observed	14.0	17.0
	Expected	15.9	15.1
2	Observed	7.0	20.0
	Expected	13.8	13.2
1	Observed	0.0	12.0
	Expected	6.1	5.9
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	39.339
p-value	0.000

Shade

		Sex	
Node		Female	Male
5	Observed	21.0	4.0
	Expected	16.9	8.1
4	Observed	16.0	7.0
	Expected	15.5	7.5
3	Observed	16.0	13.0
	Expected	19.6	9.4
2	Observed	7.0	5.0
	Expected	8.1	3.9
1	Observed	N/A	N/A
	Expected	N/A	N/A
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	5.608
p-value	0.132

Table 11. Chi-Square test for independence between node and sex of flower with respect to number of plants producing flowers of each sex in the sun and shade treatment for population B.

Sun

Node		Sex	
		Female	Male
5	Observed	17.0	5.0
	Expected	10.6	11.4
4	Observed	17.0	14.0
	Expected	14.9	16.4
3	Observed	11.0	15.0
	Expected	12.5	13.5
2	Observed	5.0	15.0
	Expected	9.6	10.4
1	Observed	0.0	5.0
	Expected	2.4	2.6
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	17.323
p-value	0.002

Shade

Node		Sex	
		Female	Male
5	Observed	6.0	10.0
	Expected	4.0	12.0
4	Observed	5.0	13.0
	Expected	4.5	13.5
3	Observed	1.0	13.0
	Expected	3.5	10.5
2	Observed	1.0	3.0
	Expected	1.0	3.0
1	Observed	N/A	N/A
	Expected	N/A	N/A
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	3.788
p-value	0.285

Table 12. Chi-Square test for independence between node and sex of flower with respect to number of plants producing flowers of each sex in the sun and shade treatment for population C.

Sun

Node		Sex	
		Female	Male
5	Observed	28.0	6.0
	Expected	17.9	16.1
4	Observed	25.0	16.0
	Expected	21.6	19.4
3	Observed	22.0	20.0
	Expected	22.1	19.9
2	Observed	14.0	24.0
	Expected	20.0	18.0
1	Observed	0.0	14.0
	Expected	7.4	6.6
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	32.551
p-value	0.000

Shade

Node		Sex	
		Female	Male
5	Observed	17.0	12.0
	Expected	17.6	11.4
4	Observed	21.0	12.0
	Expected	20.0	13.0
3	Observed	19.0	14.0
	Expected	20.0	13.0
2	Observed	14.0	8.0
	Expected	13.4	8.6
1	Observed	N/A	N/A
	Expected	N/A	N/A
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	0.386
p-value	0.943

Table 13. Chi-Square test for independence between node and sex of flower with respect to number of plants producing flowers of each sex in the sun and shade treatment for population D.

Sun

		Sex	
Node		Female	Male
5	Observed	26.0	12.0
	Expected	16.2	21.8
4	Observed	18.0	21.0
	Expected	16.6	22.4
3	Observed	14.0	25.0
	Expected	16.6	22.4
2	Observed	7.0	26.0
	Expected	14.1	18.9
1	Observed	1.0	5.0
	Expected	2.6	3.4
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	19.105
p-value	0.001

Shade

		Sex	
Node		Female	Male
5	Observed	14.0	11.0
	Expected	11.8	13.2
4	Observed	16.0	19.0
	Expected	16.6	18.4
3	Observed	8.0	13.0
	Expected	9.9	11.1
2	Observed	6.0	6.0
	Expected	5.7	6.3
1	Observed	N/A	N/A
	Expected	N/A	N/A
0	Observed	N/A	N/A
	Expected	N/A	N/A

X^2	1.543
p-value	0.672

Stalk Position: Number of Days to First Flowering Results

ANOVA was not performed on stalk data because during the course to the experiment few plants produced flowers at this position. The small sample sizes and lack of female flowers in general made ANOVA tests impractical. General patterns can be interpreted from graphs that show the number of days to maturation of the first flower of each sex at each node for each of the populations (Figure 7-10). Population A showed that nodes flowered sequentially starting with the lower nodes and that the stalks produced male flowers earlier than female flowers when both sexes appeared at a given node (Figure 7). In population B, nodes flowered sequentially, starting with the lower nodes, and the stalks produced female flowers earlier than male flowers when both sexes appeared at a given node (Figure 8). In population C nodes flowered sequentially, starting with the lower nodes; female flowers matured earlier than male flowers when both sexes appeared at a given node, except at nodes one and two where the male flowers matured slightly before the females (Figure 9). Population D showed that nodes started flowering sequentially, starting with the lower nodes, and that the stalks produced almost exclusively male flowers, except for one female flower at node four (Figure 10).

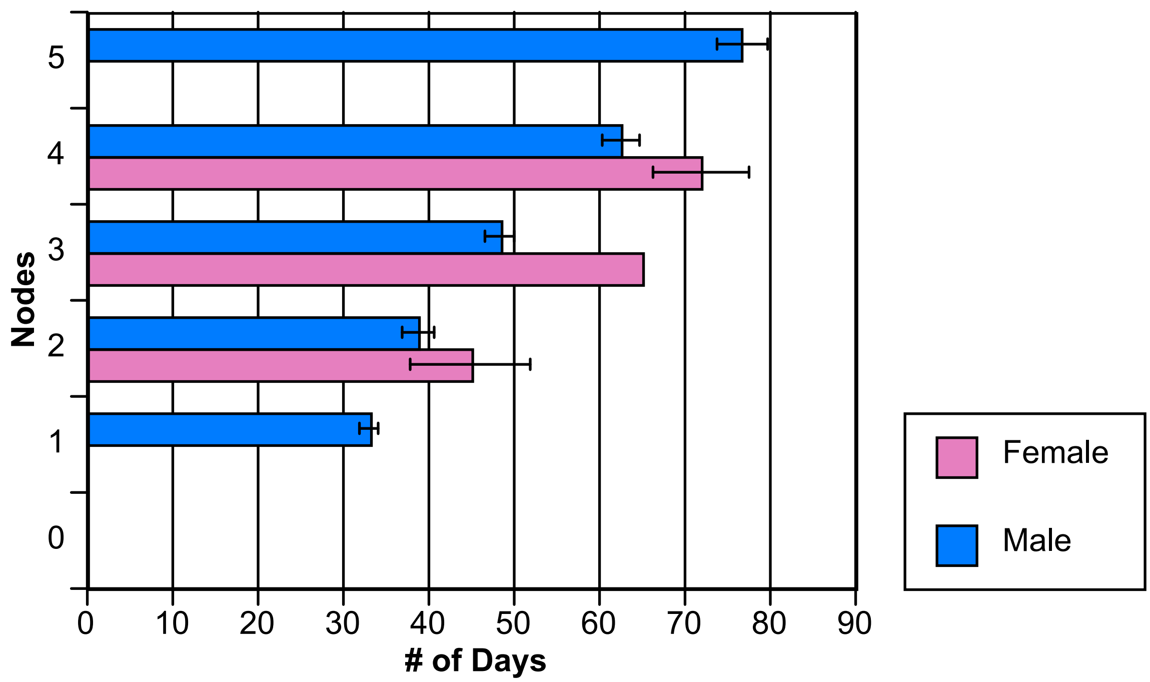

Figure 7. Number of days to maturation of the first flower of each sex at each node for population A. Bars represent $\pm$ 1 standard error.

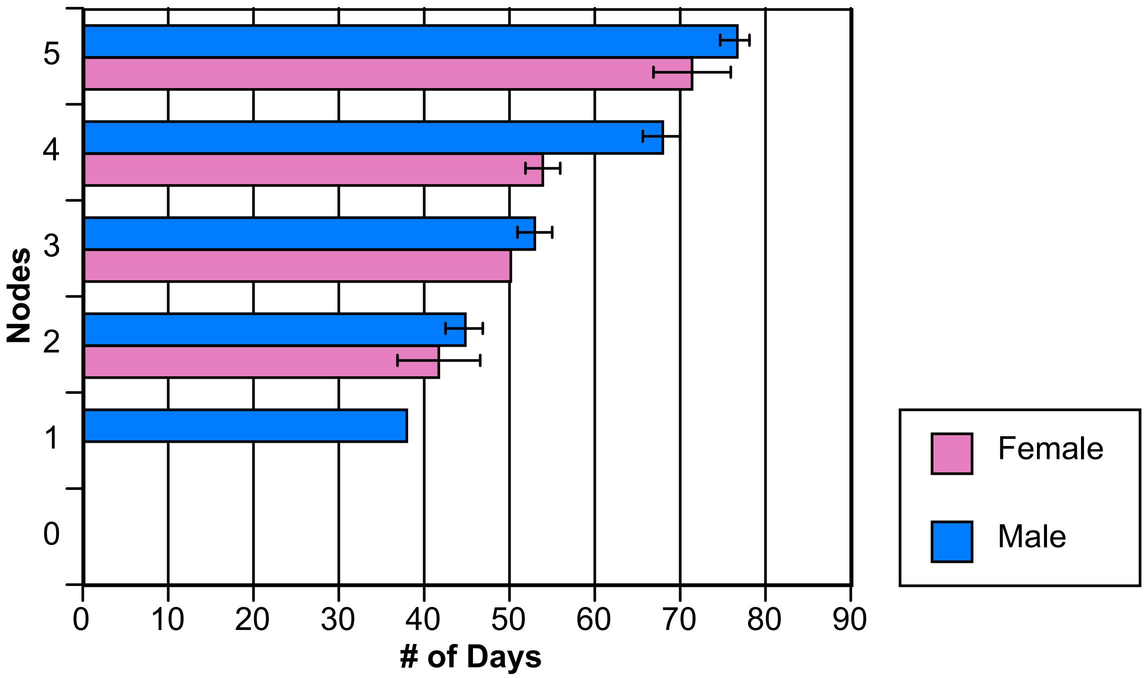

Figure 8. Number of days to maturation of the first flower of each sex at each node for population B. Bars represent + 1 standard error.

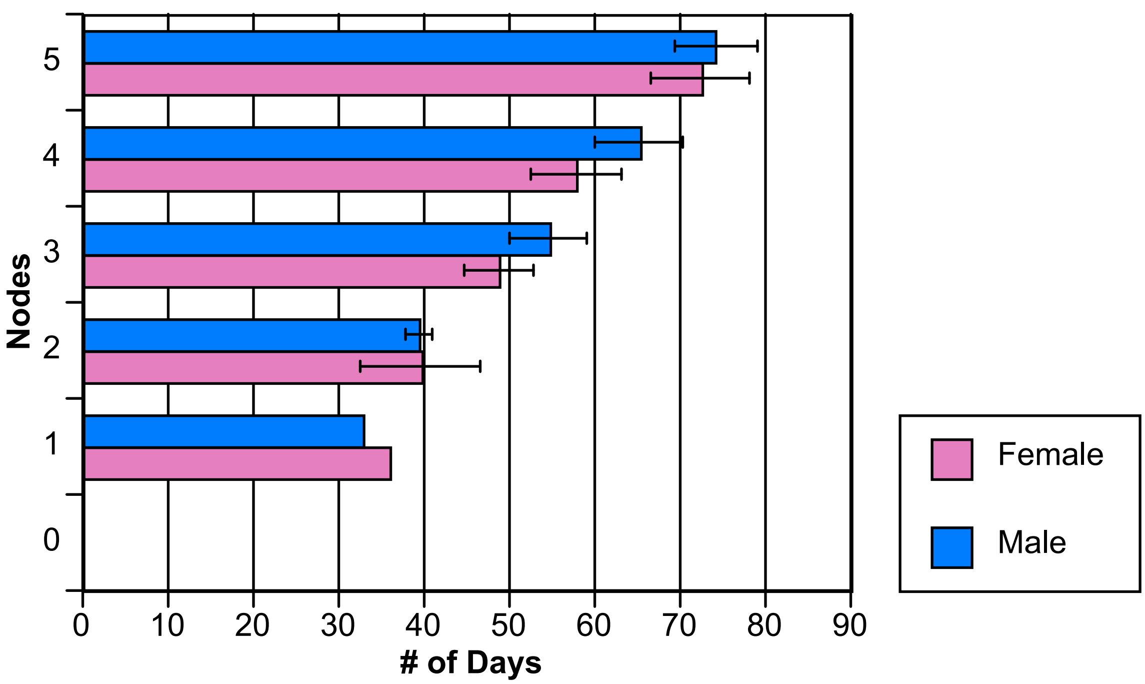

Figure 9. Number of days to maturation of the first flower of each sex at each node for population C. Bars represent + 1 standard error.

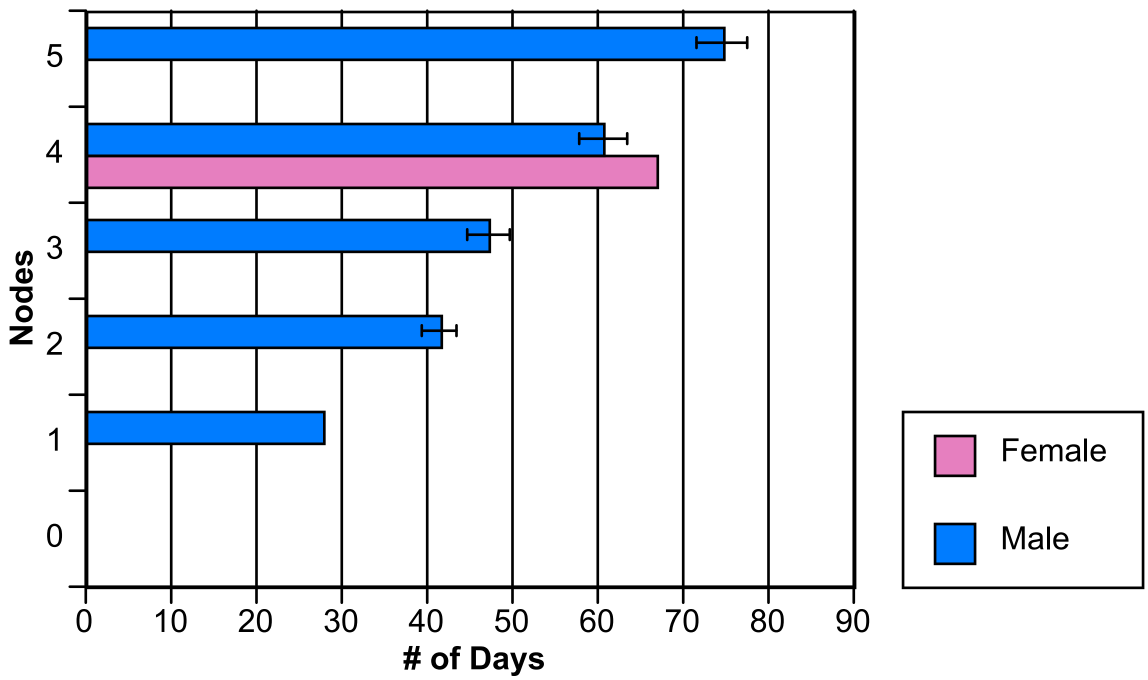

Figure 10. Number of days to maturation of the first flower of each sex at each node for population D. Bars represent ± 1 standard error.

Stalk Position: Number of Plants Producing Flowers

Chi-Square tests were performed on the number of plants producing flowers to look for significant interactions between light and sex. Flowering at individual nodes was not analyzed because few plants produced flowers on stalks at any given node. Populations A and C showed a significant light by sex interaction (Tables 14 and 16). For both populations, the number of plants producing female flowers was lower than expected in the sun, and higher than expected in the shade (Tables 14 and 16). Both populations also showed that the number of plants producing male flowers was higher than expected in the sun and lower than expected in the shade, opposite from the trend seen for the number of plants producing female flowers for both populations (Tables 14 and 16). Populations B and D did not show a significant 'light by sex' interaction. (Tables 15 and 17). Population C had many more plants producing female flowers on stalks (Table 16) than the other three populations, which had very few (Tables 14, 15, and 17).

Table 14. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population A.

Light		Sex	
		Female	Male
Sun	Observed	2.0	56.0
	Expected	5.0	53.0
Shade	Observed	5.0	18.0
	Expected	2.0	21.0

X^2	6.979
p-value	0.008

Table 15. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population B.

Light		Sex	
		Female	Male
Sun	Observed	7.0	42.0
	Expected	5.4	43.6
Shade	Observed	4.0	46.0
	Expected	5.6	44.4

X^2	0.990
p-value	0.320

Table 16. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population C.

Light		Sex	
		Female	Male
Sun	Observed	12.0	32.0
	Expected	17.6	26.4
Shade	Observed	18.0	13.0
	Expected	12.4	18.6

X^2	7.185
p-value	0.007

Table 17. Chi-Square test for independence between light and sex of flower with respect to number of plants producing flowers of each sex for population D.

		Sex	
Light		Female	Male
Sun	Observed	0.0	27.0
	Expected	0.6	26.4
Shade	Observed	1.0	18.0
	Expected	0.4	18.6

X^2	1.453
p-value	0.228

Axillary Growth Position: Number of Days to Flowering

ANOVA was not performed on the axillary growth data because during the course of the experiment, few plants produced flowers at this position. The small sample sizes and lack of female flowers in general precluded the use of ANOVA tests. General patterns can be interpreted from graphs that show the number of days to maturation of the first flower of each sex at each node for each of the populations (Figure 11-14). Population A started producing female flowers at sequential nodes, starting at node two (Figure 11). There were only two plants that produced male flowers in population A (Figure 11). Population B did not show any type of consistent pattern in the number of days to maturation of both sexes (Figure12). In population C, nodes started flowering sequentially starting with the node one, but node zero deviated from this pattern by producing mature flowers later than node one (Figure 13). Population C also showed that female flowers matured first, except at node two (Figure 13). Population D showed that the nodes started flowering sequentially starting with the node one, and the flowers are almost all female except at node one (Figure 14). Very few plants in the four populations produced flowers at the axillary growth position. In some cases only a single flowering event was observed for a given node.

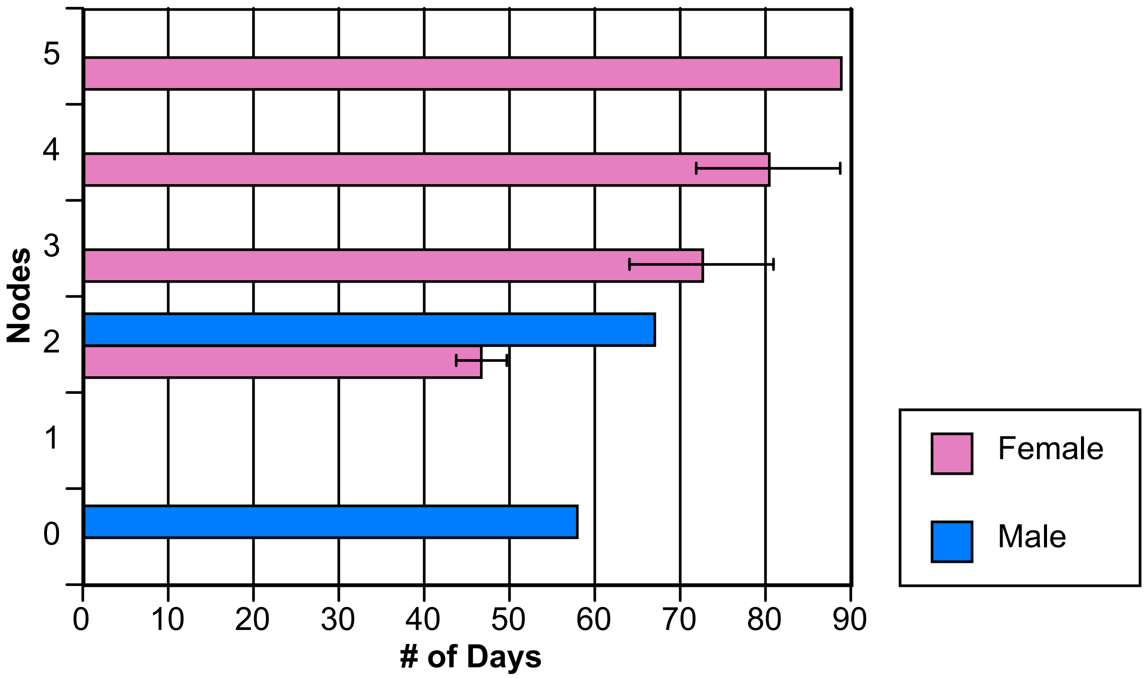

Figure 11. Number of days to maturation of the first flower of each sex at each node for population A. Bars represent ± 1 standard error.

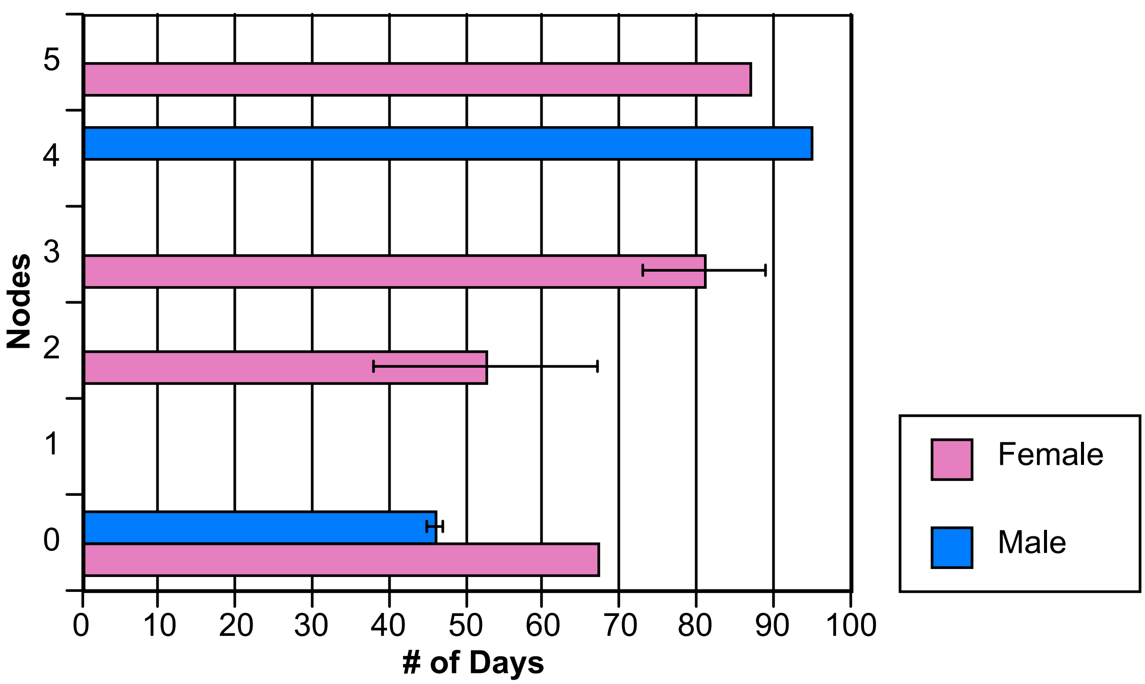

Figure 12. Number of days to maturation of the first flower of each sex at each node for population B. Bars represent ± 1 standard error.

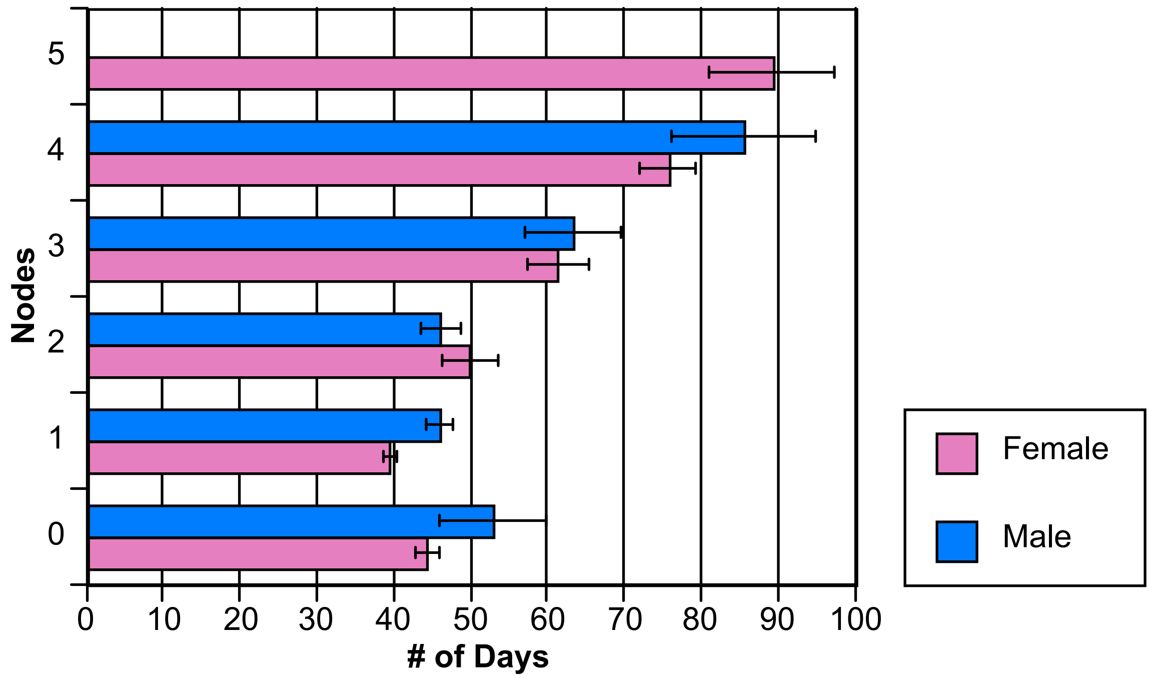

Figure 13. Number of days to maturation of the first flower of each sex at each node for population C. Bars represent ± 1 standard error.

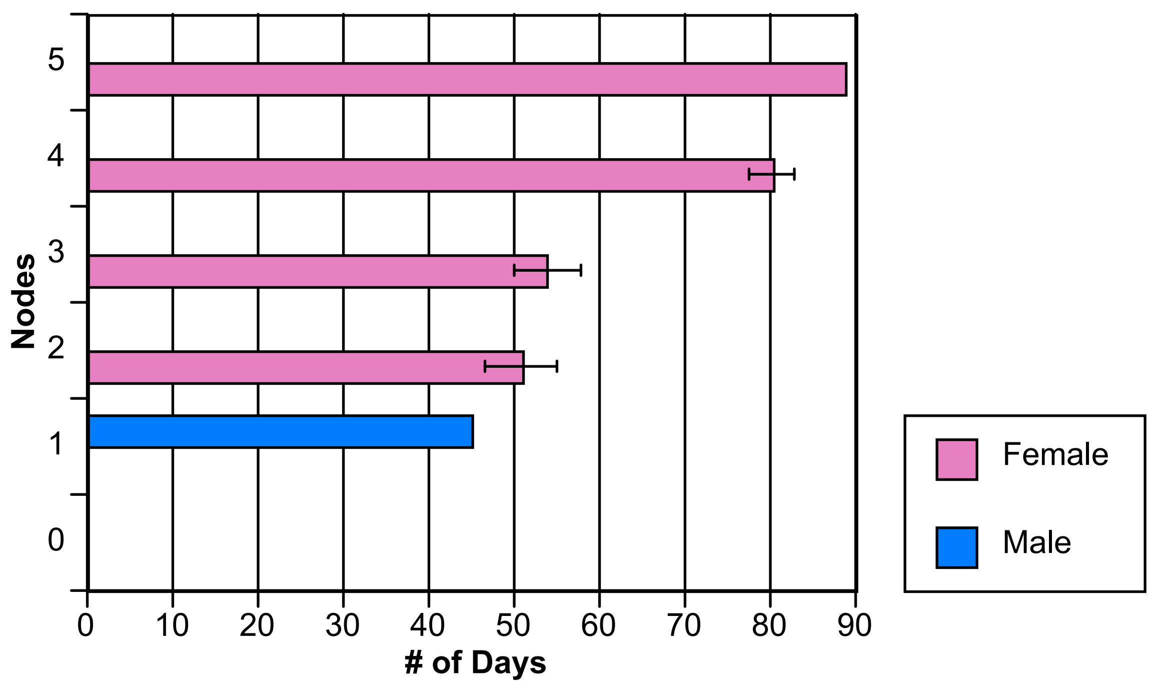

Figure 14. Number of days to maturation of the first flower of each sex at each node for population D. Bars represent ± 1 standard error.

Axillary Growth Position: Number of Plants Producing Flowers

Chi-Square tests were not performed on the axillary growth data because very few plants produced flowers at the axillary growth position making Chi-Square tests undependable. However, a comparison of the observed data and expected values for a 'light by sex' cross-tabulation shows that populations A, B, and D closely match expected values for the number of plants producing each sex of flower in each light treatment (Tables 18, 19, and 21). Population C shows deviation from the expected numbers in that in the sun there were fewer plants producing female flowers than expected and more producing males than expected and in the shade there were more plants producing female flowers than expected and fewer producing males (Table 20). Population C had many more plants producing female flowers at the axillary growth position (Table 20) than the other three populations which had very few (Tables 18, 19, and 21).

Table 18. Crosstabs results for number of plants producing each sex of flowers for both light treatments for population A.

		Sex	
Light		Female	Male
Sun	Observed	7.0	2.0
	Expected	7.4	1.6
Shade	Observed	7.0	1.0
	Expected	6.6	1.4

Table 19. Crosstabs results for number of plants producing each sex of flowers for both light treatments for population B.

		Sex	
Light		Female	Male
Sun	Observed	4.0	2.0
	Expected	4.0	2.0
Shade	Observed	2.0	1.0
	Expected	2.0	1.0

Table 20. Crosstabs results for number of plants producing each sex of flowers for both light treatments for population C.

Light		Sex: Female	Sex: Male
Sun	Observed	59.0	21.0
	Expected	62.3	17.7
Shade	Observed	22.0	2.0
	Expected	18.7	5.3

Table 21. Crosstabs results for number of plants producing each sex of flowers for both light treatments for population D.

Light		Sex: Female	Sex: Male
Sun	Observed	6.0	1.0
	Expected	6.4	0.6
Shade	Observed	4.0	0.0
	Expected	3.6	0.4

Discussion:

ANOVA results for the number of days to first flowering for the axillary position showed that plants behaved differently in each light treatment. The plants in the sun consistently matured earlier than the plants in the shade. The shaded plants were very lanky in appearance compared to the sun plants, and they were later in germination time, growth, and reaching the point of sexual maturity. The plants in both sun and shade were also flowering at sequential nodes in the axillary, stalk, and axillary growth positions starting at the lower nodes. In regards to which sex of flower matured first in each light treatment, female flowers appeared first in both the sun and shade treatments and for all of the positions. However, both the stalk and axillary growth position produced very few flowers in general and were male flower biased and female flower biased, respectively. The lack of a ‘light by sex’ interaction showed that the plants were not changing the pattern for when each sex of flower first matured in the different light conditions; the plants in the shade exhibited similar delays in maturity for both male and female flowers. Overall, the number of days to flowering results did not support the hypothesis that plants in the shade treatment will delay the onset of female flowers as an indicator of decreased allocation to female function, an hypothesis based on Pannell's (1997a) field observations. Although this hypothesis was not supported, the fact that the populations differed in which factors influenced the number of days to first flowering suggests that populations differ in flowering behavior. Populations may have evolved different evolutionary responses to shading, an environmental stress.

It was evident when observing the plants that not all plants produced flowers of both sexes at all nodes and at all positions. This lack of flowering at all nodes and position combinations may have been a consequence of arbitrarily limiting the observation period. Consequently, the plants had not reached full sexual maturity by the end of the experiment. For some of the node and position combinations only one plant in the whole population produced a flower. Thus, the mean number of days to first flowering may have been greatly influenced by small sample sizes for the populations. Analyzing the number of plants producing flowers may be a better indicator of how the plants are behaving under varying light conditions.

Chi-Square tests of independence between light conditions and number of plants producing flowers of each sex, lumping all nodes together, showed that some populations at each position exhibited a significant interaction between light and sex. The stalk and axillary growth positions were not analyzed by node because there were not enough plants producing flowers of either sex at these positions to be able to analyze individual nodes. Within the populations that showed significant interactions at each position, the contrasting light treatments differed in whether male or female flowers were more numerous than expected. For population A (axillary position), populations A and C (stalk position), and population C (axillary growth position), there were fewer than the expected number of plants producing female flowers and more than the expected number of plants producing male flowers in the sun treatment. In the shade treatment, a larger number of plants than expected produced female flowers.

When looking for significance in the node by light interaction in the axillary position, the nodes were separated and the sexes were lumped together. All of the populations showed the same pattern, with the sun treatment having more plants producing flowers at the lower nodes than was expected and having fewer plants producing flowers at the upper nodes than was expected. The shade treatment showed the opposite effect and had fewer plants producing flowers at the lower nodes and had more plants producing flowers at the upper nodes than expected. Only populations A and C actually deviated enough from the expected values to provide significant Chi-Square values. Since this was a consistent pattern seen when the nodes were separated, but the sexes were lumped together, it was important to look at how each of the sexes were influencing these results.

For the 'node by sex' interaction, where the light treatments analyzed separately, all of the populations showed a consistent pattern. In the sun treatment there were fewer plants producing female flowers at the lower nodes and more plants producing female flowers at the upper nodes than was expected. The number of plants producing male flowers showed the opposite pattern by having more than expected at lower nodes and fewer than expected at the upper nodes. This pattern showed a shift towards more plants producing female flowers at the upper nodes as the plant matured, thus becoming more female. This shift towards femaleness at later nodes was not seen in the shade treatment. The number of plants producing each sex of flower in the shade does not show any sort of nodal pattern. These results show that lumping the sexes together for the 'node by light' interaction test swamped out the individual pattern of flower production by sex in the sun treatment.

The results of the 'node by sex' Chi-Square test support the hypothesis that, in the shade, fewer plants will produce female flowers, taking resources away from female function. The fact that there was no shift towards femaleness at the upper nodes in the shade shows that the plants were behaving differently under this environmental stress. The interaction between light and sex in all three populations produced a reversal in which of the sexes was more frequent than expected in the shade as compared to the sun. The observation that population B (axillary position) differs from the other populations and positions by being female biased in the sun treatment suggests that populations may have evolved different allocation responses to changes in light conditions.

An example of how the populations may be evolving different sex allocation responses to environmental stress may be illustrated by behavior of plants in population C. Plants in this population were more highly branched and had more axillary reproductive structures than did plants in the other populations. This population also had more plants producing flowers of either sex at all of the growth positions.

The fact that female flowers appear first overall helps to explain why the plants shift their allocation away from female function in the shade. Plants in the shaded conditions have more limited resources than plants in the sun, and thus have fewer resources to allocate to reproductive function. Since female flowers and the fruits they produce are more expensive then male flowers, and in this plant species females mature first, the greatest impact on resource use by the plants should occur by shifting resources away from the more energetically costly female flowers.

The arbitrary cut-off date may have unintentionally swayed the results because there was no real way of knowing if the plants would have shown different patterns if plants had been followed to maturity. Time constraints on this project did not allow for further observations past the arbitrary cut off date. The prior study looking at the effects of shading on *Mercurialis annua* used seed count, dry fruit weight per plant biomass, and pollen production as variables to test for shading effects, which may be better indicators of sex allocation in this species than the number of days to flowering or the number of plants producing each sex of flowers (Pannell, 1997a). The previous study also harvested the plants for data collection, but this study allowed the plants to be monitored over a portion of their life cycle, producing different types of result than previously reported (Pannell 1997a).

Overall, this study helps to support the field observations that cosexual plants from androdioecious *Mercurialis annua* populations reduce their allocation to female function in the shade as compared to those growing in the full sun (Pannell 1997a). The four populations studied also were responding to shading differently and thus provide an opportunity for the evolution of different resource allocation patterns in this species. This is one of the few studies to examine the role of environmental stresses on the phenology of flower production in an androdioecious species.

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Appendix A. Means, standard errors, and sample sizes for number of days to flowering in axillary position for populations A-D, broken down by light, sex and node.

POP	LIGHT	SEX	NODE	N	Mean	Std. Error of Mean
A	sun	female	2	7	33.29	4.455
			3	14	35.36	1.041
			4	20	46.30	2.139
			5	22	58.64	1.652
		male	1	12	33.58	1.390
			2	20	38.45	.966
			3	17	44.47	1.065
			4	9	57.00	3.346
			5	2	63.00	13.000
	shade	female	2	7	46.57	1.251
			3	16	54.13	2.674
			4	16	63.50	2.703
			5	21	76.76	1.600
		male	2	5	52.80	2.888
			3	13	63.08	1.838
			4	7	74.14	2.857
			5	4	82.00	3.697
B	sun	female	2	5	32.20	1.934
			3	11	39.09	2.011
			4	17	48.12	1.946
			5	17	62.94	1.158
		male	1	5	34.20	1.744
			2	15	39.53	.910
			3	15	48.53	1.897
			4	14	59.93	2.655
			5	5	71.40	2.421
	shade	female	2	1	54.00	.
			3	1	54.00	.
			4	5	55.60	1.470
			5	6	73.83	3.371
		male	2	3	50.33	2.028
			3	13	60.00	1.905
			4	13	74.23	2.972
			5	10	84.40	3.334

POP	LIGHT	SEX	NODE	N	Mean	Std. Error of Mean
C	sun	female	2	14	28.07	1.131
			3	22	35.14	1.037
			4	25	41.88	1.531
			5	28	53.46	1.959
		male	1	14	33.00	1.011
			2	24	37.67	.906
			3	20	41.75	1.138
			4	16	49.31	2.213
			5	6	55.50	4.631
	shade	female	2	14	46.86	1.382
			3	19	52.47	2.269
			4	21	59.33	1.764
			5	17	69.71	1.504
		male	2	8	61.63	4.807
			3	14	60.93	1.542
			4	12	69.50	2.024
			5	12	83.17	2.153
D	sun	female	1	1	33.00	.
			2	7	33.71	2.437
			3	14	39.57	1.401
			4	18	50.78	2.907
			5	26	59.69	1.747
		male	1	5	35.80	.800
			2	26	39.23	1.011
			3	25	46.00	1.361
			4	21	57.14	2.242
			5	12	67.17	3.563
	shade	female	2	6	52.67	7.504
			3	8	53.63	5.165
			4	16	55.44	2.143
			5	14	63.64	2.645
		male	2	6	50.17	.749
			3	13	60.69	1.998
			4	19	71.11	2.321
			5	11	72.73	2.711