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Inheritance of photoperiod-induced flowering in three photoperiodic lines of *Aeschynomene americana* L.*

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Summary. Induction of flowering by photoperiod was studied in the parental, F_1, F_2, and reciprocal backcross generations of crosses between three photoperiod-responsive *Aeschynomene americana* L. lines. Generation means appeared additive. Analysis with Mather and Jinks' scaling tests showed little or no epistasis and indicated that an additive-dominance model was adequate. Partitioning components of variation revealed that nearly all variation was additive genetic with dominance and environmental variation negligible. An additive genetic model with two loci, each with two alleles and all alleles having equal net effect, was tested using Power's partitioning method. Results demonstrated that the model fit the data and that there is a major additive genetic system controlling flowering in these crosses, with minor genetic and environmental influences present. Selection for flowering at a desired day length should be feasible.

Key words: Photoperiodism – *Aeschynomene* – Flowering

Introduction

Aeschynomene americana L., known commonly by its genus name as aschynomene or American jointvetch, is a tropical/subtropical forage legume. Because aeschynomene is managed as a reseeding annual, a well-timed sequence of production of both herbage for forage and seed for the next year's crop is necessary. Flowering reduces vegetative production yet must occur before frost in subtropical areas in order to provide sufficient seed. To optimize the duration of both the vegetative and reproductive growth stages, an understanding of the genetics of flowering is essential. The objective of this study was to investigate the inheritance of photoperiod-induced flowering in three aeschynomene lines, in order to determine how flowering may be manipulated to maximize sustainable forage production.

Materials and methods

Three plant introductions of *A. americana* were selected for use as parents in a series of crosses. These pure-breeding parent lines were observed to be photoperiod-responsive at Gainesville, Florida, in previous years' plantings (Table 1). Line 206 was induced to flower very late in the year on short days, whereas lines 55 and 232 flowered around the autumnal equinox. Lines 55 and 232 were crossed (Hardy and Quesenberry 1984) with one another and with the short-day 206 line, yielding three different crosses. Single F_1 plants from each cross were backcrossed to both respective parents and were also allowed to self-pollinate to produce F_2 seed. Self-pollinated seed was also harvested from each parent plant. Thus, seed was available for the parent (P_1, P_2), F_1, F_2, and backcross (BC_{p1} BC_{p2}) generations.

Seeds were germinated in the greenhouse and at about 6 weeks of age seedlings were transplanted into the field at two Florida locations, Gainesville and Ft. Pierce. Gainesville, at 29°N latitude, is about 320 km north of Ft. Pierce, which is on the Atlantic coast at about 27°N latitude. At each site, plants were set out in a randomized complete block design with five replications. Each block was divided into three equal-sized units which were randomly assigned to each of the three crosses. Thus, each unit contained a family. These family units contained 16 five-plant rows, of which 2 rows were allocated to F_1, 6 rows to F_2, 3 rows to each backcross, and at least 1 row to each parent. Additional rows of 55 and 232 were planted in each block, thus their populations were higher than 206. Plants were spaced at 1.5 m with 2 m between rows. No guard rows or guard plants were included.

The day of the year at which flowering was first observed was recorded for each plant. The day of year was then converted to hours of daylight to account for differences in latitude between the two locations (Gate Research 1977).

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Table 1. Aeschynomene accessions used as parents in study of inheritance of photoperiod-induced flowering

I.D. No.	Origin	Photoperiod response and flowering date at Gainesville based on initial observations	Distinguishing characteristics
55	Florida	Photoperiod responsive Late September	Prolific foliage; upright; pubescent stem; pale yellow flower; yellow pollen
206	Panama	Photoperiod responsive short day Mid-November	Procumbent; glabrous stem; large yellow-orange flower; yellow pollen
232	Brazil	Photoperiod responsive Early October	Open growth habit; somewhat procumbent; pubescent stem

Table 2. Means and variances of various generations derived from crosses 55 × 232, 55 × 206, and 232 × 206 grown at Gainesville and Ft. Pierce

Generation	Cross	Gainesville			Ft. Pierce		
		n	$\bar{x}$	σ^2	n	$\bar{x}$	σ^2
Parent	55	74	12.32	0.008	75	12.53	0.086
Parent	232	74	12.40	0.032	75	12.36	0.180
F_1	55 × 232	48	12.34	0.013	49	12.44	0.141
F_2	55 × 232	143	12.31	0.047	146	12.28	0.160
Backcross	F_1 × 55	69	12.30	0.015	74	12.34	0.122
Backcross	F_1 × 232	70	12.37	0.041	69	12.41	0.188
Parent	55	74	12.32	0.008	75	12.53	0.086
Parent	206	47	10.55	0.010	45	10.87	0.023
F_1	55 × 206	20	11.40	0.067	24	11.39	0.009
F_2	55 × 206	149	11.42	0.190	149	11.47	0.139
Backcross	F_1 × 55	53	11.95	0.112	45	11.85	0.187
Backcross	F_1 × 206	74	10.92	0.077	70	11.22	0.055
Parent	206	47	10.55	0.010	45	10.87	0.023
Parent	232	74	12.40	0.032	75	12.36	0.180
F_1	232 × 206	50	11.47	0.013	48	11.40	0.009
F_2	232 × 206	145	11.55	0.226	145	11.58	0.164
Backcross	F_1 × 206	71	11.07	0.094	64	11.21	0.023
Backcross	F_1 × 232	74	11.93	0.145	75	11.85	0.167

Table 3. Frequency distributions of 55 × 206 various generations of cross at Gainesville. Numbers are individual plants per class (cell)

Generation	Upper class limits in h of daylight																
	10.4	10.6	10.8	11.0	11.2	11.4	11.6	11.8	12.0	12.2	12.4	12.6	12.8	13.0	n	$\bar{x}$	σ^2
P_1 (55)										9	57	8			74	12.316	0.008
P_2 (206)	3	32	11	1											47	10.551	0.010
F_1		1	–	1	–	2	16								20	11.399	0.067
F_2	1	4	11	11	18	17	47	14	8	12	5	1			149	11.417	0.190
BC_2							16	7	2	12	13	3			53	11.947	0.112
BC_2		6	28	15	10	10	5								74	10.920	0.077

Data were arrayed in frequency distributions of 0.2-h intervals for each cross at each location and for crosses with locations pooled. Generation means and variances were calculated and were employed in Mather's scaling tests (Mather 1949; Mather and Jinks 1971) to test for epistasis and the adequacy of an additive-dominance model. Estimates of genetic variability (V), components of variance (E, environmental variance, H, dominance variance; D, additive variance), and number of effective factors (K_1) were calculated using Mather and Jinks' method of partitioning the components of variation (Mather 1949; Mather and Jinks 1971). Powers' partitioning method was employed to further analyze these data and to test a proposed genetic model (Powers 1955, 1963; Powers and Locke 1950; Leonard et al. 1957).

Results and discussion

In cross 55 × 232, parent lines and their F_1 were similar in their flowering behavior. Means for all generations were approximately the same (Table 2). Frequency distributions showed an equal range of flowering for all generations with no modality in the backcrosses or F_2s. The similarity of flowering habit of the parental lines and generations of offspring suggested that 232 and 55 carry the same alleles for flowering induction by photoperiod.

When data from the crosses 55 × 206 and 232 × 206 were displayed in frequency distributions, no obvious modality was observed in segregating populations. An example is cross 55 × 206 (Table 3). Generation means, however, showed a pronounced additive trend. The absence of discrete segregating classes and the additive nature of the means suggested that the character could be either quantitative, with a series of minor genes which together had additive effects, or it could be of simpler inheritance, where major gene action was additive and gene numbers were few, but class distinction was not obvious.

The results of Mather's scaling tests (Mather and Jinks 1971) are shown in Table 4. At Gainesville, both crosses 55 × 206 and 232 × 206 gave insignificant values for A, B, and C, indicating that the additive-dominance model was appropriate. However, the Ft. Pierce data yielded three *t*-tests which were different from zero ($P<0.05$). This may have been due to the greater variability observed at Ft. Pierce, where two blocks were planted in a field of higher fertility than the other three. Attempts were made to account for this block effect through the error variances. When the data from both locations were combined, again two tests showed a significant deviation from zero ($P<0.05$), both in the 232 × 206 cross. But these did so only by a very slight margin and, in fact, are not different from zero at $P=0.01$.

Table 4. Mather's scaling tests applied to crosses 55 × 206 and 232 × 206 to test adequacy of additive-dominance model

Gainesville	Ft. Pierce	Loc. combined
55 × 206		
$t_A=$ 1.693	$t_A=-1.613$	$t_A=-0.242$
$t_B=-1.296$	$t_B=$ 3.466**	$t_B=$ 0.798
$t_C=$ 0.017	$t_C=-2.251$*	$t_C=-1.493$
232 × 206		
$t_A=-1.179$	$t_A=$ 2.080*	$t_A=$ 2.005*
$t_B=-0.184$	$t_B=-0.576$	$t_B=-0.557$
$t_C=$ 1.872	$t_C=$ 1.831	$t_C=$ 2.447*

*, ** $t_{0.05}=1.96$ and $t_{0.01}=2.58$, respectively at infinite degrees of freedom

Table 5. Six-parameter scaling tests applied to crosses 55 × 206 and 232 × 206 to test for non-allelic interactions and to estimate components of means

Cross	Location	*m*	(*d*)	(*h*)	(*i*)	(*j*)	(*l*)
55 × 206	Gainesville	11.368 ± 0.181**	0.883 ± 0.009[a]	−0.387 ± 0.441	0.066 ± 0.181	0.289 ± 0.111**	−0.135 ± 0.286
	Ft. Pierce	11.343 ± 0.163**	0.834 ± 0.020[a]	0.197 ± 0.187	0.266 ± 0.185	−0.410 ± 0.147**	−0.242 ± 0.312
	Loc. Combined	11.390 ± 0.122**	0.860 ± 0.014[a]	0.200 ± 0.308	0.180 ± 0.122	0.060 ± 0.087**	−0.200 ± 0.191
232 × 206	Gainesville	11.670 ± 0.039**	−0.926 ± 0.012[a]	−0.296 ± 0.472	−0.196 ± 0.160	0.189 ± 0.010**	0.093 ± 0.283
	Ft. Pierce	11.795 ± 0.169**	−0.747 ± 0.027[a]	−0.479 ± 0.413	−0.182 ± 0.167	0.212 ± 0.115	0.086 ± 0.250
	Loc. Combined	11.745 ± 0.132**	−0.835 ± 0.016[a]	−0.435 ± 0.318	−0.200 ± 0.858	0.150 ± 0.084	0.130 ± 0.192

** $t_{0.01}=2.58$ at ∞ degrees of freedom
[a] for (*d*) $t_{0.01}=2.61$ at 120 degrees of freedom

Table 6. Components of variance of crosses 55 × 206 and 232 × 206 obtained by Mather's partitioning method

Cross	Location	*E*	*H*	*D*	h_n^2	No. effective factors
55 × 206	Gainesville	0.028	0	0.324	0.85	2.40
55 × 206	Ft. Pierce	0.039	0.039	0.078	0.40	8.92
55 × 206	Loc. combined	0.044	0.030	0.111	0.35	6.66
232 × 206	Gainesville	0.018	0	0.416	0.92	2.06
232 × 206	Ft. Pierce	0.071	0	0.186	0.57	3.00
232 × 206	Loc. combined	0.053	0	0.280	0.73	2.49

Table 7. Chi-squares of test for homogeneity between F_2 data and theoretical distributions

Cross	Location	Chi-square	*df*	Probability
55 × 206	Gainesville	3.23	5	0.70–0.50
55 × 206	Ft. Pierce	7.22	4	0.10–0.05
55 × 206	Loc. combined	12.02	6	0.10–0.05
232 × 206	Gainesville	16.30	5	<0.01
232 × 206	Ft. Pierce	6.13	4	0.20–0.10
232 × 206	Loc. combined	12.03	7	0.20–0.10

However, since some tests were found significant, an indication of the possibility of epistasis, the data was further analyzed with a six-parameter model to test for non-allelic interaction and to estimate components of means (Mather and Jinks 1971). Results are presented in Table 5. In both crosses at both locations, the additive component (*d*) was highly significant, whereas dominance (*h*) was not significant in any case. In general, there was little evidence of epistasis; neither the additive × additive (*i*) nor the dominance × dominance (*l*) components showed any significance in either cross. Epistasis was indicated in the additive × dominance component (*j*) of 232 × 206 at Gainesville and 55 × 206 at both locations. These results were not in complete agreement with the three-parameter scaling test where both crosses fit the additive-dominance model at Gainesville. The inconsistent and infrequent indications of epistasis suggested that if it was a component, it was of minor consequence. However, results of both the three- and six-parameter models consistently indicated a large and highly significant additive component in both crosses at each location and with locations combined.

Partitioning of the components of variation (Mather and Jinks 1971) demonstrated that the environmental variance (*E*) fo both crosses was relatively low, hence a large proportion of the total variation was genetic (Table 6). The dominance component (*H*) of the genetic variation was negligible, being zero in four cases. Thus, most of the genetic variation was additive. Additive variance does not necessarily imply additive gene action, but Falconer (1981) states that "if we find that all the genotypic variance is additive, we can conclude that the genes show neither dominance nor epistasis." Therefore, the additive variance observed was due mainly to additive gene action. Estimates of the number of effective factors were also calculated (Table 6). Although there were two higher estimates for 55 × 206, most numbers ranged between two and three, which suggested qualitative inheritance.

Test of additive model with Powers' partitioning method

With an indication that inheritance of flowering response was possibly controlled by an additive major genetic system, the data were tested for fit to a two-gene model, each locus with two alleles which act additively and with equal effect. Since no obvious modality was observed in the segregating generations' frequency distributions, Powers' partitioning technique was employed to analyze the data, following the example of F_2 partitioning by Sage and de Isturiz (1974).

In the hypothesized two-locus, additive model, the F_2 should segregate into a ratio of 1:4:6:4:1. Chi-square probabilities listed in Table 7 demonstrated that the genetic model fits the F_2 data. All probabilities were 0.05 or greater except for 232 × 206 at Gainesville. When the tails of the distribution were combined to give ten or more plants per cell, the calculated Chi-square of this cross had a probability of <0.01.

The general procedure for the backcross analysis was the same as that of the F_2. Plants were expected to segregate phenotypically in a 1:2:1 ratio. As can be seen in the summary of backcross Chi-squares in Table 8, there were six tests that fit the genetic model. These results lend strong support to the conclusion reached, after analysis of the F_2, that the model was adequate.

However, there were also six backcrosses with $P<0.01$, indicating lack of homegeneity. The lack of consistently good fit between the theoretical and observed distributions of the backcrosses may be due to the fact that the total range of the parents is too narrow to easily identify classes. Backcrosses are of narrower range than F_2s and contain fewer genotypes; all genotypes lie between the F_1 and the recurrent parent. For example, if 206 is of genotype AABB and 55 is A′A′B′B′, possible genotypes in (55 × 206) × 206 are AABB, A′AB′B, and

Table 8. Chi-squares from test of homogeneity between backcross data and theoretical distributions. (Note: Chi-squares and probabilities in parentheses are calculated by combining two adjacent cells to account for environmental error)

Cross	Location	Chi-square	*df*	Probability
(55 × 206) × 55	Gainesville	14.14 (1.20)	3 (2)	<0.01 (0.70–0.50)
(55 × 206) × 55	Ft. Pierce	3.12	4	0.70–0.50
(55 × 206) × 55	Loc. combined	24.89 (7.73)	5 (4)	<0.01 (0.10)
(55 × 206) × 206	Gainesville	0.93	2	0.50–0.30
(55 × 206) × 206	Ft. Pierce	0.57	1	0.50–0.30
(55 × 206) × 206	Loc. combined	2.79	2	0.30–0.20
(232 × 206) × 206	Gainesville	7.42	3	0.10–0.05
(232 × 206) × 206	Ft. Pierce	11.05	1	<0.01
(232 × 206) × 206	Loc. combined	3.39	3	0.50–0.30
(232 × 206) × 232	Gainesville	16.29 (2.58)	3 (2)	<0.01 (0.20–0.10)
(232 × 206) × 232	Ft. Pierce	18.81 (12.46)	5 (4)	<0.01 (0.05–0.01)
(232 × 206) × 232	Loc. combined	16.41 (4.41)	5 (3)	<0.01 (0.20–0.10)

A'ABB or AAB'B. The latter genotypes differ from the F_1 genotype by only one allele. The effect of that allele may not be easily or consistently discerned over a relatively short range of flowering dates. In addition, environmental factors could blur the distinction between genotypes. It is well established that environment can affect the expression of major genes controlling flowering (Ison 1983; Murfret 1973, 1977; Swindell and Poehlman 1978). Environmental variation caused by flooding, fertility, and rabbit herbivory were observed. Also, it is possible that other minor genes influence flowering. All these factors could cause similar but different genotypes (differing by one allele) to be classified as the same phenotype, i.e., fall into the same cell on the frequency distribution. Pooling two adjacent cells of the frequency distribution, as practiced by Powers (1955) and Sage and de Isturiz (1974), compensated for this error. When this was done, a total of 11 of the 12 backcrosses supported the hypothesized model (values in parentheses in Table 8).

Conclusion

Generation means of 55 × 232 indicated that response to photoperiod was controlled by the same alleles in these two lines. Initial observations of the generation means of crosses 55 × 206 and 232 × 206 indicated that genes controlling photoperiod-induced flowering were additive in effect. Testing the data with Mather's scaling tests supported this contention; most tests showed that the additive-dominance model was adequate. Further testing revealed little or no dominance variance and suggested that the number of effective factors was few, most frequently two. Hence, it was proposed that inheritance of flowering in these crosses might be controlled at two loci, each with two alleles and all alleles acting additively with equal effect.

Powers' partitioning method was used to test the genetic model. F_2 data sets fit the model well in five of the six analyses performed; Chi-square probabilities were generally in the 0.20–0.30 range. Backcrosses also supported the model in half of the data sets analyzed. Since most evidence fit the model, it was suspected that the six backcrosses that did not fit were affected by minor genetic or environmental effects (flowering dates of the parents being too similar) and the arbitrary nature of the frequency distribution cells. Minor manipulation of the data resulted in 11 of 12 backcrosses tested fitting the model. Despite the fact that a few analyses did not support the hypothesis, the majority of the evidence demonstrated that flowering in these two crosses may be controlled primarily by an additive genetic system with two loci, each with two alleles of equal effect. Selection for a desired flowering date, therefore, should be successful.

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