GENOTYPE-ENVIRONMENT INTERACTION AND DEVELOPMENTAL REGULATION IN *ARABIDOPSIS THALIANA*

III. INBRED LINES; ANALYSIS OF RESPONSE TO PHOTOPERIOD

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1. INTRODUCTION

THE two environmental variables which appear most frequently to control floral initiation in a specific manner are daylength and temperature. In previous papers (Westerman and Lawrence, 1970; Westerman, 1970), the response of inbred lines of *Arabidopsis thaliana* to temperature has been observed to be very diverse when measured in terms of flowering time characters. For all characters, however, a large proportion of the response is accounted for by a linear regression on to the environmental variable. Furthermore, the predominant mode of developmental regulation on flowering time and leaf number appears to be such that the expression of the character is advantageously stabilised, while with respect to height most families manifest varying amounts of flexibility.

In this paper we shall investigate whether this behaviour is a regular feature of the material by considering the response of inbred lines when *photoperiod* is used as the environmental variable. Although there has been little general work on the photoperiodic behaviour of *Arabidopsis*, it is known to be a quantitative long-day plant with a very low critical daylength (Laibach, 1943; Gregory and Hussey, 1953; Ratcliffe, 1961). Previous studies have also demonstrated that flowering time characters interact with photoperiod (Harer, 1951; Barthelmess, 1967).

The developmental phenotype with reference to photoperiod as the environmental variable has therefore been analysed genetically by means of a diallel cross between the same seven lines used in the previous paper.

2. MATERIAL AND METHODS

Details of the seven lines used as parents are given in table 1 of the previous paper. A full diallel set of crosses was constructed from these lines, and the 42 F_1 's and seven selfed progeny were raised in three controlled environment cabinets set at photoperiods of 12, 16 and 20 hours respectively, with a constant temperature of 20° C. In each environment, five seeds per family were sown on agar medium in each of two independently randomised blocks (see Westerman and Lawrence, 1970, for culture method).

The primary characters scored were flowering time, height and basal leaf number, while reproductive performance was measured as the number of siliquae produced by each plant.

3. RESULTS

The number of days from sowing to the first plant flowering in each cabinet was 31, 19 and 17 for the 12-, 16- and 20-hour photoperiods

TABLE 1

Mean flowering time of the families of a diallel set of crosses at each of three photoperiods

12 hours								16 hours							
	1	2	3	4	6	7	$\bar{r}$		1	2	3	4	6	7	$\bar{r}$
1	32.80	30.80	32.10	40.40	27.62	29.90	33.00	10.20	8.55	8.20	8.90	7.75	6.85	8.90	
2	34.10	35.03	35.10	34.90	28.90	27.92	33.75	14.80	8.23	8.35	7.80	8.70	6.70	9.32	
3	40.30	44.60	38.80	37.00	32.62	36.90	36.54	9.40	16.87	9.10	10.90	9.40	9.10	9.72	
4	37.97	38.40	38.00	37.28	29.20	30.70	35.15	8.60	8.70	8.27	9.00	8.85	8.20	8.81	
6	26.45	28.92	29.00	26.40	23.10	25.50	27.46	6.70	7.30	8.60	7.50	8.23	5.90	7.89	
7	30.80	31.25	34.65	33.37	28.67	27.90	30.29	6.70	7.60	9.30	10.00	7.50	7.30	7.70	

20 hours							
	1	2	3	4	6	7	$\bar{r}$
1	3.50	2.80	4.28	5.00	4.50	3.78	4.15
2	2.30	3.62	5.62	4.18	3.43	3.64	4.01
3	4.62	5.60	5.76	6.30	5.32	5.70	5.53
4	4.98	5.50	5.90	5.50	5.57	5.50	5.34
6	4.60	3.50	5.00	4.75	6.30	4.00	5.12
7	5.90	4.25	6.50	5.40	8.17	5.57	5.33

$\bar{r}$ = Array mean.

TABLE 2

Mean height at flowering time of diallel families at each photoperiod

12 hours								16 hours							
	1	2	3	4	6	7	$\bar{r}$		1	2	3	4	6	7	$\bar{r}$
1	38.18	36.30	61.70	62.30	55.42	30.70	47.57	60.40	64.68	84.70	87.50	70.00	62.33	70.13	
2	42.50	40.35	61.40	60.00	49.80	32.15	47.98	58.47	61.22	75.32	70.80	92.90	59.10	69.60	
3	66.30	64.30	79.48	76.65	67.00	45.50	64.86	80.80	85.07	97.70	101.30	92.00	83.80	89.35	
4	58.12	62.80	73.00	77.57	61.12	43.40	63.66	74.80	75.20	96.10	97.50	94.55	78.30	86.39	
6	47.22	50.30	64.00	66.60	66.60	41.20	56.33	77.20	78.70	97.60	91.20	76.53	77.60	82.80	
7	33.90	35.47	39.47	44.80	40.17	24.20	36.26	60.30	52.50	80.10	71.87	68.75	58.83	67.69	

20 hours							
	1	2	3	4	6	7	$\bar{r}$
1	41.70	45.70	55.07	53.30	51.20	42.85	48.23
2	44.70	42.17	56.87	57.55	52.58	41.64	49.23
3	55.50	59.60	84.30	86.10	76.78	59.20	70.63
4	57.87	56.33	93.30	87.12	83.48	49.60	69.63
6	54.60	55.10	80.20	68.50	58.60	45.60	61.76
7	34.60	36.35	56.30	55.30	55.83	34.35	45.50

$\bar{r}$ = Array mean.

TABLE 3

Mean leaf number of diallel families at each photoperiod

12 hours								16 hours							
	1	2	3	4	6	7	$\bar{r}$		1	2	3	4	6	7	$\bar{r}$
1	12.70	13.50	12.30	12.50	12.62	14.60	12.89	7.60	7.50	7.10	8.00	8.25	7.43	8.01	
2	12.80	12.93	12.60	11.80	12.20	13.55	12.86	10.00	7.43	7.43	7.20	7.20	8.40	8.24	
3	13.30	13.20	11.40	11.10	12.45	13.50	12.48	7.50	10.73	6.80	7.40	7.90	7.10	7.67	
4	12.45	13.30	11.90	11.50	12.18	13.70	12.24	9.10	8.80	7.10	7.60	8.52	8.60	8.02	
6	12.50	12.35	12.50	11.60	11.70	12.30	12.30	7.80	8.20	8.00	8.00	8.02	7.60	7.90	
7	12.70	13.18	14.12	13.40	13.50	14.70	13.66	8.20	8.60	8.20	8.25	7.25	7.62	7.91	

20 hours							
	1	2	3	4	6	7	$\bar{r}$
1	7.80	7.30	7.98	8.10	7.70	8.55	8.18
2	7.35	8.12	8.00	8.12	8.52	8.40	8.28
3	9.13	8.50	7.80	7.80	8.23	9.20	8.45
4	8.80	9.68	7.90	7.88	9.05	9.80	8.56
6	8.30	7.80	8.60	8.75	8.60	9.20	8.57
7	9.35	9.50	10.50	8.93	9.50	9.37	9.31

 $\bar{r}$ = Array mean.

TABLE 4

Mean silique number of diallel families at each photoperiod

12 hours								16 hours							
	1	2	3	4	6	7	$\bar{r}$		1	2	3	4	6	7	$\bar{r}$
1	17.08	17.30	15.90	15.00	19.75	23.50	17.81	13.80	16.48	11.30	14.10	13.00	15.33	14.15	
2	16.70	18.58	15.30	16.00	19.50	20.78	17.98	16.87	12.77	12.85	15.50	12.90	15.10	14.50	
3	13.60	15.70	11.58	12.18	18.15	18.30	15.26	10.40	11.44	13.30	12.90	16.50	13.40	13.26	
4	14.05	15.80	12.60	12.00	18.42	21.90	15.70	14.20	15.60	12.93	13.45	12.80	17.60	14.65	
6	22.42	20.87	19.00	18.80	22.40	27.10	21.30	14.60	14.70	16.70	16.20	19.37	19.00	15.91	
7	21.37	20.65	19.28	19.62	26.75	33.60	23.86	15.90	17.00	14.10	17.12	15.75	17.42	16.26	

20 hours							
	1	2	3	4	6	7	$\bar{r}$
1	14.90	14.30	16.40	16.10	15.30	17.70	15.82
2	12.33	16.54	15.00	15.22	14.15	16.66	15.43
3	15.35	16.60	13.60	15.00	16.28	15.80	15.14
4	14.45	14.95	14.50	14.50	14.37	16.50	15.18
6	17.00	14.50	14.60	15.33	18.30	18.90	16.24
7	21.12	18.40	15.00	16.70	17.84	22.95	18.38

 $\bar{r}$ = Array mean.

respectively. Losses due to poor germination were low (5 per cent.). A further 22 per cent. of the plants, however, had not flowered by the time the experiment was terminated, after approximately 75 days of scoring. With two exceptions only, these plants were progenies of parent 5; this array was in consequence extremely poorly represented at all photoperiods. For the purposes of analysis, array 5 was therefore omitted from the data. It is not clear why these families have failed to flower in this experiment. Some 10 per cent. of the plants of this line, however, failed to flower in the first experiment of this series (Westerman and Lawrence, 1970), though all did so in the second experiment (Westerman, 1970). Thus the performance of line 5 changed considerably from the first to the second experiment, while that of other lines was highly repeatable.

The family and array means at each photoperiod are presented in tables 1-4. As in the previous papers, the square root transformation has been applied to all data prior to analysis.

(a) *Average phenotype*

The Hayman (1954) analyses for all four characters are shown in table 5. While the additive mean square in each case is the largest item with respect to the average phenotype, significant non-additive effects are also present for all characters except height. Reciprocal differences occur much less frequently than in the previous diallel set of crosses (Westerman, 1970,

TABLE 5

Hayman analyses of variance of the average phenotype. Entries are mean squares

Source	d.f.	FT	HT	LN	SN
<i>a</i>	5	1.4577***	33.3517***	0.1292***	3.3036***
<i>b</i>	15	0.1071**	0.1118	0.0324**	0.1063**
<i>b</i> ₁	1	0.0052	0.1388	0.1168**	0.0675
<i>b</i> ₂	5	0.0920	0.1702	0.0117	0.0927
<i>b</i> ₃	9	0.1268**	0.0765	0.0345**	0.1182**
<i>c</i>	5	0.4219***	0.0771	0.0669***	0.0881
<i>d</i>	10	0.0726	0.0807	0.0235	0.0395
<i>t</i>	35	0.3351***	4.8465***	0.0486***	0.5414***
Environments	2	248.6485***	49.5603***	12.0116***	4.3560***
<i>E</i> × <i>a</i>	10	0.7265***	0.7610***	0.0646***	0.5512***
<i>E</i> × <i>b</i>	30	0.0593	0.2550*	0.0124	0.1095***
<i>E</i> × <i>b</i> ₁	2	0.0142	0.1257	0.0038	0.0216
<i>E</i> × <i>b</i> ₂	10	0.0287	0.2206	0.0089	0.1222**
<i>E</i> × <i>b</i> ₃	18	0.0813	0.2884*	0.0153	0.1122***
<i>E</i> × <i>c</i>	10	0.0466	0.0913	0.0140	0.0384
<i>E</i> × <i>d</i>	20	0.0590	0.2110	0.0171	0.0225
<i>E</i> × <i>t</i>	70	0.1527***	0.2913***	0.0214**	0.1376***
Blocks	1	0.0997	4.6147***	0.0381	0.1502
<i>B</i> × <i>t</i>	35	0.0373	0.1777	0.0290***	0.0363
<i>E</i> × <i>B</i>	2	0.2488**	0.2391*	0.0532*	0.4018**
<i>E</i> × <i>B</i> × <i>t</i>	70	0.0617	0.1740	0.0138	0.0678**
Replicates	—	0.0512 (791)	0.1653 (782)	0.0134 (791)	0.0468 (782)

FT = flowering time, HT = height, LN = leaf number, SN = siliqua number.

a result which can be attributed to a difference in crossing technique. In the construction of the present set of crosses, each parental line was represented by a single plant, used both as male and female parent. In the

FIG. 1.— W_r/V_r graphs for flowering time, leaf and silique number with respect to the average phenotype. In the top-left corner, the parental means are ranked according to their position on the graph.

previous diallel, on the contrary, one plant from each line was used as a male and another as a female parent. This difference in technique would not normally be expected to have this consequence with homozygous parents. There is some evidence, however, that reciprocal differences in this species are not a regular feature of the inheritance of the characters in question, but are generated by small differences in the parental environment (Lawrence, personal communication).

The non-additive variation in respect of flowering time, leaf and siliqua number can be examined in greater detail by means of Wr/Vr graphs (Jinks, 1954); the values of Wr and Vr for each array have been corrected for environmental effects in the usual manner. The regressions of Wr on Vr are all significantly different from zero (fig. 1), and furthermore the slopes do not differ from unity, except at the 5 per cent. probability level with respect to flowering time. The relationship between parental means and the order of the points on the graph indicates that dominance is ambidirectional for flowering time and siliqua number, while for leaf number dominance appears to be in the direction of a greater number of leaves.

The ambidirectional dominance observed for siliqua number would, on the argument that the genetic architecture of a character indicates the type

TABLE 6
Hayman analysis of the linear and non-linear components of the developmental phenotype for siliqua number

Source	Linear		Non-linear	
	d.f.	M.S.	d.f.	M.S.
<i>a</i>	5	0.7593***	20	0.1546***
<i>b</i>	15	0.1211***	60	0.0666*
<i>b</i> ₁	1	0.0011	4	0.0136
<i>b</i> ₂	5	0.1672**	20	0.0878*
<i>b</i> ₃	9	0.1088*	36	0.0607
<i>c</i>	5	0.0205	20	0.0383
<i>d</i>	10	0.0350	40	0.0427
<i>t</i>	35	0.1733***	140	0.0683**
Replicates	782	0.0468	782	0.0468

of selection to which the character has been subject, lead us to conclude that intermediate rather than high siliqua number is most advantageous. There are, however, two main reasons why this argument carries little force in the present circumstances. Firstly, dominance is incomplete, both in this experiment and the previous one (Westerman, 1970). Secondly, and of greater consequence, the dominance properties detected here relate to non co-adapted gene complexes, the parents concerned having been obtained from diverse origins. Thus, while in other situations this argument would have much greater relevance, for the present we may continue to regard high siliqua number as synonymous with high fitness.

Turning now to the inheritance of the developmental phenotype, only additive effects interact with the environment for all primary characters. With respect to siliqua number, however, the control of the developmental phenotype depends upon both additive and non-additive variation.

(b) *Developmental phenotype*

In the previous paper, the nature of the genotype-environment interaction displayed by each character was investigated by partitioning the interaction into linear and non-linear components (Perkins, 1970), and subjecting each component to a genetic analysis. In the present data, however, only additive variation is responsible for the genotype-environment interactions manifested by the three primary characters, and thus the main point of

this analysis is to ascertain the proportion of the response that is linear in nature. In fact, linear effects account for 90, 66 and 48 per cent. of the total variation ascribable to genotype-environment interaction with regard to flowering time, height and leaf number respectively.

FIG. 2.—The relationship of mean silique number and the response of the three primary characters.

The analysis of the developmental phenotype for silique number is shown in table 6. The inheritance of the linear component is controlled by additive and non-additive variation, while effectively only additive effects interact in a non-linear fashion with environments; 86 per cent. of the interaction is attributable to a linear response to the environment. The regression of Wr on Vr with respect to the linear component of interaction is not significant.

(c) *Relationship of primary characters with siliqua number*

The use of the linear regression coefficient as a measure of the response to environmental change has, in earlier papers, been justified by the predominant linear nature of performance with respect to environment. The approach used, whereby the developmental phenotype of a primary character is related to the average phenotype of a fitness character, in no way depends on the use of this response metric; any convenient measure of variability over environments will suffice. In the present experiment, the linear response accounts for just less than half of the genotype-environment interaction displayed by leaf number and, accordingly, we must employ a measure of response which considers both the linear and non-linear components of the developmental phenotype. For this character, therefore, mean siliqua number has been plotted against the variance relating to differences between environments and blocks, with five degrees of freedom, of each family (fig. 2). Under these circumstances, the diagram is divided into four parts by the mean siliqua number of the parents, and by the average variance of the parental lines. The relationship of leaf number with siliqua number can then be ascertained in the usual manner.

The interpretation of the diagram for flowering time (fig. 2) is clear. All the points lie on a significant regression line running from the top-left to the bottom-right quarter; that is, the mode of developmental regulation with respect to flowering time appears to be such that stability is advantageous. Parents 6 and 7 are relatively stable, while the remaining parents are instable, a low average number of siliquae being associated with a large change in time of flowering over environments.

An examination of the diagram for height (fig. 2) reveals that, with the exception of line 6, all points fall on a significant regression line running from the top-right to the bottom-left quarter; in other words, all families manifest some degree of flexible response, parent 7 being the most flexible line. On the other hand, line 6 is highly stable, in that it produces an above average number of siliquae and changes little with respect to height over environments.

Turning finally to the graph of mean siliqua number against the variance over environments for leaf number, we find that no clear pattern emerges; the regulation of development of this character may be considered therefore to be more diverse than that of the other primary characters.

4. DISCUSSION

The results from this experiment cannot be compared directly with those from the previous paper (Westerman, 1970) because of the failure of array 5 to flower. To facilitate this comparison, this array was therefore omitted from the previous diallel set of crosses also, and for both experiments the components of mean squares were estimated with respect to each character.

From table 7 it is clear that, although the total phenotypic variance is always larger in the diallel set of crosses using temperature as the environmental variable, the estimates of the variance between lines (σ_L^2) for both experiments are in general in good agreement; for all primary characters; the response of the lines over the ranges examined is in fact slightly greater for temperature than for photoperiod. Examination of the estimated mean

squares reveals that the characters fall into two distinct groups. For flowering time and leaf number over 90 per cent. of the total variance is environmental, the components for between lines and interaction accounting for approximately equal amounts of the remaining variation. Furthermore, these characters interact more with temperature than with photoperiod. For height and siliqua number, the environmental variance is a much smaller proportion of the total, and the lines $\times$ environments components are larger when photoperiod is the environmental variable.

We may now consider the nature of the developmental phenotype manifested in response to the environmental variables, temperature and photoperiod. The percentage of the response to temperature which is

TABLE 7

Estimated components of mean squares

Character	Environmental variable	σ_L^2	σ_E^2	$\sigma_{L \times E}^2$
FT	Temperature	0.0103	0.4474	0.0131
	Photoperiod	0.0081	0.5527	0.0084
HT	Temperature	0.1542	0.1157	0.0113
	Photoperiod	0.1037	0.0722	0.0429
LN	Temperature	0.0024	0.0233	0.0033
	Photoperiod	0.0010	0.0267	0.0013
SN	Temperature	0.0064	0.0186	0.0019
	Photoperiod	0.0151	0.0102	0.0085

linear is 99, 86, 96 and 37 per cent. of the total variation ascribable to genotype-environment interaction with respect to flowering time, height, leaf and siliqua number respectively; that is, for all three primary characters the interaction is almost entirely a linear function of the environmental values. In contrast, 90, 66, 48 and 86 per cent. of the interactions with photoperiod are a linear function of the environmental values for flowering time, height, leaf and siliqua number, respectively; that is, only the interaction of flowering time and siliqua number with photoperiod is predominantly linear. Thus flowering time alone has a linear response to both environmental variables.

The final point concerns the mode of developmental regulation of the primary characters. When either temperature or photoperiod is used as the environmental variable, the optimum level of variability is low with respect to flowering time, and is high with respect to height. In their different ways, therefore, the development of these characters apparently remains the same whether the environmental variable involves differences in temperature or in photoperiod. It appears therefore that, in this material, in the environments here examined, the mode of regulation is a property of the character as such, and is not narrowly dependent on the environment. Flowering time, then, appears to be a character whose optimum is brought about by the stabilisation of the expression of the genes concerned; this will lead as far as possible to synchronous flowering, and indeed evidence from populations in the wild supports this thesis. The development of height on the other hand, appears to be able to respond in an adaptive manner to variation of the environment.

5. SUMMARY

1. The average and developmental phenotype of the same seven lines used in the previous paper has been examined using photoperiod as the environmental variable. Three primary characters, flowering time, height and leaf number, were scored, while reproductive performance was measured as the number of siliquae produced by each plant.

2. The inheritance of the average phenotype with respect to height is controlled only by additive variation, while significant non-additive effects are also present for the remaining characters.

3. All characters manifest genotype-environment interaction, and furthermore for flowering time and siliqua number this interaction is almost wholly a linear function of the environmental values.

4. Only additive effects interact with the environment for the three primary characters, but both additive and non-additive variation are involved for siliqua number.

5. The optimum level of variability in response to environmental change is low with respect to flowering time, and high with respect to height.

6. These results are compared with those from a previous paper.

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