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

## I. INBRED LINES; DESCRIPTION

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### 1. Introduction

The ease with which the investigation of the genetic structure of a population can be accomplished depends on the genetic architecture of the character in question. In the case of characters determined by a few genes of large effect, the species may often be investigated directly in its natural habitat. Metrical characters, however, appear to be determined by many genes whose individual effects are small and are readily modified by a variable environment. The partition of genetic and environmental effects can usually be accomplished in these circumstances only if the material is investigated indirectly, in a suitably designed laboratory experiment.

The need to proceed in this way, however, can raise a particularly troublesome interpretative difficulty. The degree of confidence with which we can infer the properties of the genes determining a metrical character in natural habitats, from observations of their properties in a laboratory environment, depends on the extent to which the effect of genotype and environment are independent in respect of their determination of the phenotype. Such confidence can be high where genotype and environment act independently. Where, on the other hand, gene expression varies from one environment to another it is difficult, if not impossible, to infer its significance in natural populations without a detailed knowledge of the interaction of each genotype with every environment found in the natural habitat of the species in question. Viewed in this way, the presence of genotype-environment interaction in the material under investigation serves only to complicate the analysis of population structure.

The widespread occurrence of genotype-environment interaction, however, raises the important question of its evolutionary significance. There are in principle, as Thoday (1953) has pointed out, two ways in which an individual may react to a variable environment. On the one hand, an individual's genotype may be such that its development is buffered against environmental variation, the same adaptive phenotype being produced in a range of environments. On the other hand, its genotype may be such that it can develop different phenotypes in different environments, each phenotype being better adapted to the environment that evokes it than any other. Thoday regards both of these situations as manifestations of developmental flexibility. Others, who have sought to distinguish these alternatives have regarded the first situation as developmental stability (Mather, 1953), phenotypic stability (Lewis, 1954), developmental homeostasis (Lerner, 1954; Dawson, 1968), canalisation (Waddington, 1942) and autoregulation (Schmalhausen, 1949). The second situation has been

variously referred to as phenotypic plasticity (Salisbury, 1940), adaptive plasticity (Mather, 1955), developmental flexibility (Levins, 1963) and individual adaptability (Cook and Johnson, 1968). Bradshaw (1965) refers to all environmentally induced variation as plasticity, whether it is environmental variation as such or genotype-environment interaction *sensu stricto*. Despite the fact that this profuse terminology reflects to some extent the varied interests of the investigators concerned, there appears to be a need for a consistent and unambiguous definition of the situations we have in mind. We propose therefore the terms *developmental stability* and *developmental flexibility* in respect of the first and the second situations discussed earlier. The further term *developmental regulation* can then be used when we wish to refer to both of these adaptive situations in a generic fashion. With

TABLE 1

*Types of developmental regulation*

| | Genotype determines *same* phenotype in different environments | Genotype determines *different* phenotype in different environments | |
|---|---|---|---|
| Average fitness high | Developmentally stable | Developmentally flexible | Development well regulated |
| Average fitness low | Developmentally inflexible | Developmentally unstable | Development poorly regulated |

their respective antithetical situations, developmental instability and developmental inflexibility we then have the four basic relationships between the phenotypic variability of the primary character and fitness shown in table 1. Developmental stability and developmental flexibility are, as Lewontin (1957) has pointed out, the developmental analogues of the population concepts of mono- and poly-morphism and, indeed, may represent alternative evolutionary strategies to the latter, though the circumstances in which one or other is the outcome is far from clear.

This theory of the evolutionary role of genotype-environment interaction in natural populations is relatively simple. It is less easy to apply this theory to an actual population, however, for we encounter four main difficulties whenever we attempt to do so. Firstly, fitness cannot, of course, be measured; the best we can do is to measure one or other of the components of reproductive performance of the material under investigation. Though this approximation nearly always involves an arbitrary decision, it is particularly so in the present circumstances. Thus, secondly, only selectively important characters can be expected to have had their development well adjusted by selection. Yet if such a character is selectively important it is, by definition, a component of fitness. We find ourselves, therefore, concerned here with a comparison between the variability of a primary character and that of some measure of reproductive performance where both are components of fitness. Thirdly, wherever natural selection has adjusted the expression of the genes determining the primary character it has done so in relation to the environments found in the natural habitats of the species

concerned. Yet we know little enough about the major environmental factors affecting populations of any species, so that again our choice of environment must necessarily be somewhat arbitrary. The fourth and last difficulty concerns the choice of material. Any analysis of the significance of environmental variation with respect to a character requires, if it is to be informative, that the material should comprise lines or populations whose response to a changing environment is sufficiently diverse as to include at least two of the basic situations shown in table 1. Though this requirement appears to be self-evident, a species may well have evolved the same pattern of developmental regulation in many populations—an interesting state of affairs, but one which would be singularly difficult to analyse without reference to a population which had evolved differently.

Despite these difficulties there is an extensive literature on developmental regulation (see Bradshaw, 1965). Few of these cases involve measures of fitness, though in some it can hardly be doubted that the observed response is in fact adaptive. An example of the former is the work of Allard and Workman (1963) on the $F_1$ progeny of a pair of homozygous lines of the lima bean, which outyield the lines in poor seasons, but not in good ones. With regard to the latter kind of case, inbred lines of *Drosophila melanogaster* are well known to be less fecund and less fertile than their crossbred progenies. Mather (1953) has shown that the stability of such lines with respect to sternopleural chaeta number is less than that of their $F_1$ progeny. Since it is possible to select for both high and low stability it is clear that this developmental character is under genetic control (Mather, *loc. cit.*; Thoday, 1955; Reeve, 1960).

Convincing evidence of developmental flexibility is less common. Northern European strains of perennial ryegrass (*Lolium perenne*) are sensitive to temperature, relative to strains from the Mediterranean, such that with the onset of winter there is a marked reduction of the rate of leaf expansion in the former but not in the latter. The flexibility of the northern strains in this respect is clearly adaptive, because it is associated with a higher probability of survival (Cooper, 1963). Cook and Johnson's (1968) studies of variability in populations of the heterophyllous *Ranunculus flammula* and Bjorkman and Holmgren's (1963) work on leaf expansion and light intensity in *Solidago virgaurea* are two cases among several which provide suggestive, if not compelling, evidence of developmental flexibility.

So few cases hardly provide sufficient evidence for any general theory concerning the environmental circumstances which favour the evolution of developmental stability rather than developmental flexibility—or, indeed, vice versa. But we can argue that whenever a population of a species has regularly to contend with a heterogeneous environment, a developmentally flexible response is likely to constitute the optimum adaptational strategy. In other words, we expect such an environment to cause disruptive selection, not on the frequencies of the genes determining the average phenotype of the character in question, as is presumably the case in the evolution of a polymorphism, but rather on the frequencies of the genes controlling the developmental phenotype of the character. Less regular variation of the environment would then be countered best by a system of developmental stability. We lack, however, evidence of the type of case which would be most informative in this and indeed other problems of developmental regulation, namely, a species which had evolved developmental stability

with respect to the expression of a character in some of its populations and developmental flexibility with respect to the same character in others. An investigation of the environments evoking these different patterns of developmental regulation, together with an analysis of their genetic determination, could tell us much about the advantage of this, developmental, polymorphism relative to the better understood genetic polymorphisms.

It was with these considerations in mind that the present investigation was begun with thirty-three inbred lines of *Arabidopsis thaliana*, an annual species of flowering plant which is widely distributed throughout Europe and elsewhere. Previous investigations (Barthelmess, 1967; Jones, unpublished) have shown that several heritable characters associated with flowering time, including flowering time itself, display considerable amounts of genotype-environment interaction. Observation of the species, furthermore, both in its natural habitats (well-drained open situations and gardens) and in the laboratory, show that, as in many other weedy species, its morphology is capable of considerable modification, particularly with regard to size. It is therefore, in Bradshaw's (1965) terminology, clearly a plastic species.

According to Ratcliffe (1961) the species behaves as a winter annual in part of its range in the United Kingdom and as a summer annual elsewhere. Investigation of material from British populations shows that most individuals require a variable period of vernalisation before they will flower (Jones, unpublished). It is clear, therefore, that temperature plays an important role in the life-cycle of the species and it is, accordingly, the environmental variable chosen for study here.

The last point concerns the breeding system of the species. It has been widely assumed in the past that *Arabidopsis* is an obligate inbreeder (Muller, 1961; Laibach, 1965; Ratcliffe, *loc. cit.*) and there is no doubt that isolated plants in the glasshouse will set seed freely and autogamously. More recent evidence suggests, however, that a certain, as yet undetermined, proportion of outcrossing occurs in natural populations of the species (Jones, 1968 and unpublished). Any attempt to ascertain the evolutionary role of genotype-environment interaction in this species must therefore do so in the context of populations of partially outbreeding individuals. It is desirable, however, that preliminary investigations of genotype-environment interaction should be confined to inbred lines because, as is well known, their biometrical analysis pose fewer problems than that of material of unknown genetic status.

## 2. Materials and methods

Details of the thirty-three inbred lines raised in this experiment are shown in table 2. Twenty-one lines were obtained from the Laibach collection (Robbelen, 1965), nineteen of these being wild-type and two (18 and 26) being homozygous for a mutant gene, hairless. Eight lines originated from an experiment concerning radiation-induced polygenic mutation (Lawrence, 1968*a*, *b*); these also are wild-type. Two lines were obtained from natural populations in England (7) and France (8). Lines 39 and 40 are homozygous for known major mutants.

The lines were grown in three chest-type " Prestcold " controlled environment cabinets set at temperatures of 15°, 20° and 25° C. Lighting

is supplied in these cabinets by twelve 4-ft. " Westinghouse " SHO/CW fluorescent tubes and three 60-W tungsten strip-lamps. In all cabinets light intensity was adjusted to approximately 13,000 lm./m.$^2$, this being measured at the level of the seedling plant inside the test-tubes in which

TABLE 2

*The inbred lines*

| Line No. | Name | Country of origin | Source |
|---|---|---|---|
| 1 | Enkheim | Germany | 1** |
| +2 | Eifel | Germany | 1** |
| 3 | $S_2E$ | — | 1 |
| 4 | $A_3L_2$ | — | 1 |
| 5 | Estland | U.S.S.R. | 1** |
| 6 | $C_3L_2$ | — | 1 |
| 7 | Henley-in-Arden | England | 2 |
| +8 | Maine | France | 1 |
| 9 | $S_1L_2$ | — | 1 |
| 10 | $C_2L_1$ | — | 1 |
| +11 | Langridge | — | 1 |
| 12 | $C_3E$ | — | 1 |
| +13 | Limburg | Germany | 1** |
| 14 | $A_1E$ | — | 1 |
| 15 | $S_3L_1$ | — | 1 |
| +16 | Landsberg-1 | Germany | 3** |
| 17 | Bologna-1 | Italy | 3** |
| 18 | Coimbra-1* | Portugal | 3** |
| +20 | Le Mans-2 | France | 3** |
| 22 | Palermo-1 | Italy | 3** |
| 23 | Burghhaun | Germany | 3** |
| 24 | Eifel-6 | Germany | 3** |
| 25 | Gückingen | Germany | 3** |
| +26 | Wilna-2* | U.S.S.R. | 3** |
| 27 | Oystese | Norway | 3** |
| 28 | Estland-1 | U.S.S.R. | 3** |
| 29 | Enkheim-2 | Germany | 3** |
| 33 | Pitztal-2 | Germany | 3** |
| 34 | Antwerp-1 | Belgium | 3** |
| 35 | Göttingen | Germany | 3** |
| 37 | Dijon | France | 3** |
| 39 | *stellula*-1* | — | 4 |
| 40 | *apetala** | — | 4 |

* Major mutant lines; lines 18 and 26 are glabrous.
** Laibach collection.
\+ The seven lines shown in fig. 1.
1—Dr C. W. Lawrence, Wantage.
2—Dr J. Hill, Aberystwyth.
3—Dr G. Robbelen, Göttingen, Germany.
4—Dr A. D. McKelvie, Aberdeen, Scotland.

they were housed. Daylength was similarly held constant at 16 hours. Humidity, though not controlled automatically, was maintained at a minimum of 80 per cent. R.H.

The plants were grown in 16 × 150 mm. " Pyrex " test-tubes on 7·5 ml. of asceptic agar medium (Langridge, 1957; Brown and Smith, 1964; Lawrence, C. W., personal communication). Seed was sterilised in a 1 : 1 mixture of ethanol and hydrogen peroxide (20 vols.) for 10 minutes and then

washed thoroughly in sterile distilled water before being sown singly in each test-tube by placement on the surface of the agar medium with a fine paint-brush. Asepsis was maintained during the earlier, pre-flowering stage of the experiment by plugging the test-tubes with dental cotton. The test tubes were held in the cabinets in wooden blocks at a density of approximately 20 per dm.$^2$. At the onset of flowering in each cabinet, the cotton plugs were withdrawn from all test-tubes and about half the lights switched off to maintain an approximately constant light intensity throughout the course of the experiment.

Ten seeds of each line were sown in each of two independently and completely randomised blocks in each cabinet. Each plant in the experiment was scored with respect to its flowering time (days), height at flowering time (mm.) and basal leaf number, these being regarded as the primary characters of the investigation. Day 1 on the flowering time-scale was determined by the first plant to flower in the experiment, which turned out to be an individual in the 25° C. environment, a plant which came into flower 14 days after the experiment was sown. Scoring was terminated in each environment 40 days after flowering had commenced in that cabinet, that is, on days 59, 52 and 41 in the 15°, 20° and 25° C. cabinets respectively. Reproductive output was measured by counting the number of ripe siliquae (fruits) produced by each of five plants in each line, block and cabinet, that is, half the plants in the experiment.

Some difficulty was experienced because of poor germination, developmental accidents and the occurrence of late-flowering plants in some lines. The poor germination was due to the seed being two years of age, which is older than is desirable in a species with otherwise excellent germination. On germination, the cotyledons of some of the seedlings became trapped in the agar. Since this frequently causes a considerable delay to the growth of the plant, all such individuals have been excluded from the data presented here. Losses due to both these causes amounted to just over 20 per cent. A further 3 per cent. of the plants in the experiment had not flowered when scoring was terminated. These too were excluded from the data, as none of the lines concerned appear to require vernalisation.

## 3. Results

### (i) *Analysis of variance*

The line means with respect to each of the three primary flowering time characters are shown in table 3 and those for siliqua number in table 4. Fig. 1 shows the means of seven lines plotted against temperature. It is, unfortunately, not practicable to show the means of all thirty-three lines in this way. The seven lines chosen for this purpose illustrate the general features of the experiment, and, furthermore, are the subject of a more detailed examination of genotype-environment interaction to be presented in a later paper.

Turning now to these data, there is little doubt that temperature has a marked effect on the expression of all characters, height increasing with temperature, the other characters displaying a negative relationship in this respect. Since the lines chosen for this experiment comprise a quota sample of those in the Laibach collection we are hardly surprised to find that there are pronounced differences between these too. At the same time,

their response to a change of temperature is not uniform. The flowering time of line 8, for example, changes less over the range of temperatures studied than does that of line 26, their situation being reversed in respect of siliqua number. There is thus little doubt that in respect of their response to different temperatures, the lines display genotype-environment interaction. Furthermore, since the rank we assign to a line in terms of the magnitude of its response to environmental variation varies from one character to another, it is also clear that this response is a property of the character, rather than a general property of the line in question.

We may now turn to the analyses of variance of these data. Two procedural points require mention here. First, preliminary analysis revealed an approximate linear relationship between line mean in each environment and block and the within block variance around that mean. All the data were accordingly transformed by taking the square root of the original scores. Though this resulted in a very considerable improvement, the within block variances remained heterogenous, both as regards comparison between lines and between environments. There is, indeed, very little we can do about this, because it is, of course, a manifestation of the very phenomenon the experiment was designed to investigate, albeit operating at another level.

The second point concerns the unequal numbers of observations resulting from losses mentioned earlier. In many circumstances this would indicate a requirement for a three-way analysis of variance with unequal numbers. In the present case, however, all effects are fixed (the blocks by virtue of a constant orientation in the cabinets). The analyses shown in table 5 have therefore been performed on the means of each line in each environment and block, the replicate sum of squares having been divided by the harmonic mean of the numbers of observations entering these means; that is, these analyses are unweighted.

The results of these analyses of variance confirm the impression gained from the examination of line means. Their chief purpose, however, is to enable us to ascertain the proportion of the total variance, with respect to each character in the experiment, that is ascribable to comparisons of interest, particularly that concerning genotype-environment interaction. Firstly, though some of the block comparisons are significant, they are for no character an important source of variance. Secondly, and of more interest, the magnitudes of the genotype-environment interaction variance components are, at first sight, surprisingly small, being 7, 9, 20 and 4 per cent. for flowering time (FT), height (HT), leaf number (LN) and siliqua number, respectively. However, thirdly, the magnitude of the line components are also not large, being 16, 26, 20 and 7 per cent. respectively.

Two conclusions follow from these observations. The first is that, relative to additive genetic effects, genotype-environment interaction is an important source of variance with respect to all four characters in this experiment, the latter amounting to 44, 35, 100 and 57 per cent. of the former. The second conclusion concerns one of the interpretative difficulties discussed earlier, namely, the degree of confidence which we can attach to inferences about the genetic structure of natural populations from our observations of samples taken from these and raised in a laboratory environment. If these figures, which refer of course to laboratory inbred lines, are typical of the magnitude of the genotype-environment interaction occurring

TABLE 3

*Numbers of plants and line means for flowering time (FT), height (HT) and leaf number (LN). Number in brackets show late-flowering plants excluded from the data*

15° *C. environment*

| Line | 1 | 2* | 3 | 4 | 5 | 6 | 7 | 8* | 9 | 10 | 11* | 12 | 13* | 14 | 15 | 16* | 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 17 | 17 | 18 | 18 | 18 | 19 | 11 (4) | 19 | 16 | 2 (15) | 19 | 13 | 19 | 18 | 19 | 12 (2) | 2 | |
| FT | 23·1 | 27·6 | 35·9 | 44·2 | 39·2 | 45·3 | 50·7 | 27·8 | 45·3 | 57·0 | 41·2 | 37·1 | 36·3 | 35·9 | 34·2 | 54·6 | 43·0 | |
| HT | 11·2 | 4·4 | 26·9 | 29·9 | 33·4 | 33·5 | 29·5 | 5·8 | 32·2 | 1·5 | 40·5 | 37·9 | 27·2 | 37·7 | 27·8 | 28·1 | 22·5 | |
| LN | 9·3 | 12·1 | 11·8 | 11·7 | 12·8 | 13·1 | 14·4 | 12·7 | 8·6 | 18·5 | 12·3 | 8·6 | 12·2 | 10·8 | 11·6 | 18·3 | 13·5 | |

| Line | 18 | 20* | 22 | 23 | 24 | 25 | 26* | 27 | 28 | 29 | 33 | 34 | 35 | 37 | 39 | 40 | Total and means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 10 | 16 | 7 (1) | 13 | 13 | 15 | 16 | 11 (3) | 17 | 16 | 13 | 18 | 16 | 18 | 18 | 19 | 493 (25) |
| FT | 27·0 | 30·1 | 36·7 | 41·1 | 24·6 | 27·7 | 48·3 | 53·9 | 45·7 | 31·0 | 29·5 | 24·4 | 32·0 | 34·3 | 26·9 | 29·1 | 37·0 |
| HT | 21·1 | 21·4 | 36·3 | 10·8 | 22·8 | 11·0 | 10·4 | 49·9 | 47·0 | 14·4 | 14·8 | 10·5 | 25·3 | 22·8 | 10·6 | 2·0 | 23·1 |
| LN | 10·1 | 11·4 | 11·7 | 12·1 | 8·9 | 11·8 | 15·6 | 16·1 | 13·1 | 13·4 | 11·9 | 8·3 | 12·8 | 11·5 | 11·2 | 12·1 | 12·2 |

20° *C. environment*

| Line | 1 | 2* | 3 | 4 | 5 | 6 | 7 | 8* | 9 | 10 | 11* | 12 | 13* | 14 | 15 | 16* | 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 19 | 15 | 19 | 19 | 18 | 19 | 18 | 16 | 17 | 20 | 17 | 11 | 17 | 19 | 19 | 13 (2) | 13 (3) | |
| FT | 12·5 | 16·7 | 18·8 | 23·7 | 21·8 | 20·3 | 30·4 | 15·8 | 29·8 | 30·6 | 20·6 | 20·6 | 19·9 | 19·6 | 20·6 | 38·5 | 38·5 | |
| HT | 44·6 | 12·9 | 32·7 | 30·1 | 47·2 | 25·9 | 43·6 | 13·0 | 38·6 | 9·0 | 42·7 | 51·6 | 36·8 | 50·0 | 36·1 | 39·4 | 46·2 | |
| LN | 6·7 | 10·2 | 8·5 | 10·5 | 9·1 | 8·9 | 13·2 | 9·6 | 8·2 | 13·7 | 9·9 | 7·6 | 8·9 | 8·5 | 9·3 | 17·4 | 15·5 | |

| Line | 18 | 20* | 22 | 23 | 24 | 25 | 26* | 27 | 28 | 29 | 33 | 34 | 35 | 37 | 39 | 40 | Total and means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 9 | 14 | 3 (9) | 16 | 4 | 14 | 18 | 16 | 17 | 19 | 16 | 17 | 12 | 17 | 15 | 19 | 515 (14) |
| FT | 19·2 | 20·7 | 16·3 | 21·5 | 15·8 | 16·9 | 22·1 | 33·3 | 24·6 | 19·4 | 16·1 | 15·1 | 19·5 | 20·2 | 15·0 | 16·5 | 21·5 |
| HT | 24·4 | 33·4 | 53·3 | 18·3 | 28·5 | 32·9 | 20·7 | 65·2 | 51·1 | 22·4 | 26·4 | 23·7 | 33·7 | 41·2 | 17·5 | 6·1 | 33·3 |
| LN | 9·3 | 10·6 | 7·3 | 10·1 | 6·8 | 10·1 | 14·0 | 14·6 | 10·9 | 12·0 | 11·2 | 6·9 | 11·3 | 10·8 | 8·9 | 11·1 | 10·4 |

TABLE 3—*Continued*

25° *C. environment*

| 2 R 2 Line | 1 | 2* | 3 | 4 | 5 | 6 | 7 | 8* | 9 | 10 | 11* | 12 | 13* | 14 | 15 | 16* | 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 18 | 18 | 20 | 18 | 19 | 20 | 18 | 17 | 18 | 20 | 18 | 14 | 18 | 17 (1) | 20 | 13 | 4 (10) | |
| FT | 3·8 | 7·4 | 7·3 | 7·9 | 7·7 | 8·5 | 16·6 | 7·9 | 18·7 | 12·1 | 7·1 | 7·6 | 6·6 | 7·1 | 6·9 | 25·2 | 36·8 | |
| HT | 50·3 | 52·9 | 66·6 | 51·8 | 70·1 | 62·2 | 55·1 | 47·2 | 42·3 | 31·2 | 69·3 | 75·6 | 64·2 | 78·2 | 59·8 | 58·5 | 71·5 | |
| LN | 4·2 | 6·8 | 6·3 | 6·9 | 6·4 | 7·7 | 10·3 | 6·4 | 8·9 | 9·7 | 6·1 | 6·6 | 6·5 | 6·1 | 6·1 | 11·9 | 12·5 | |

| Line | 18 | 20* | 22 | 23 | 24 | 25 | 26* | 27 | 28 | 29 | 33 | 34 | 35 | 37 | 39 | 40 | Total and means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 8 | 17 | 3 (3) | 16 | 4 | 13 | 18 | 14 | 18 | 18 | 13 | 16 | 12 | 18 | 14 | 20 | 512 (14) |
| FT | 18·1 | 9·9 | 17·0 | 10·2 | 7·5 | 7·9 | 5·7 | 12·2 | 8·6 | 10·1 | 6·4 | 7·4 | 15·8 | 8·6 | 8·1 | 8·1 | 10·8 |
| HT | 36·0 | 58·4 | 56·3 | 32·9 | 60·0 | 60·9 | 47·0 | 57·6 | 63·4 | 51·7 | 58·3 | 53·3 | 43·6 | 72·4 | 45·7 | 36·6 | 55·8 |
| LN | 11·1 | 6·8 | 9·7 | 8·0 | 5·8 | 7·9 | 6·3 | 8·9 | 6·9 | 9·4 | 6·7 | 5·4 | 11·0 | 6·7 | 7·8 | 7·9 | 7·7 |

*Line means*

| Line | 1 | 2* | 3 | 4 | 5 | 6 | 7 | 8* | 9 | 10 | 11* | 12 | 13* | 14 | 15 | 16* | 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 54 | 50 | 57 | 55 | 55 | 58 | 47 (4) | 52 | 51 | 42 (15) | 54 | 38 | 54 | 54 (1) | 58 | 38 (4) | 19 (13) | |
| FT | 13·1 | 17·2 | 20·7 | 25·3 | 22·9 | 24·7 | 32·6 | 17·2 | 31·3 | 33·2 | 23·0 | 21·8 | 20·9 | 20·9 | 20·6 | 39·4 | 39·4 | |
| HT | 35·4 | 23·4 | 42·1 | 37·3 | 50·2 | 40·5 | 42·7 | 22·0 | 37·7 | 13·9 | 50·8 | 55·0 | 42·7 | 55·3 | 41·2 | 42·0 | 46·7 | |
| LN | 6·7 | 9·7 | 8·9 | 9·7 | 9·4 | 9·8 | 12·6 | 9·6 | 8·6 | 14·0 | 9·4 | 7·6 | 9·2 | 8·4 | 9·0 | 15·9 | 13·9 | |

| Line | 18 | 20* | 22 | 23 | 24 | 25 | 26* | 27 | 28 | 29 | 33 | 34 | 35 | 37 | 39 | 40 | Grand total and grand means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 27 | 47 | 13 (13) | 45 | 21 | 42 | 52 | 41 (3) | 52 | 53 | 42 | 51 | 40 | 53 | 47 | 58 | 1520 (53) |
| FT | 21·4 | 20·2 | 23·3 | 24·3 | 16·0 | 17·5 | 25·4 | 33·1 | 16·3 | 20·2 | 17·3 | 15·6 | 22·4 | 21·0 | 16·7 | 17·9 | 23·1 |
| HT | 27·2 | 37·7 | 48·6 | 20·7 | 37·1 | 34·9 | 26·0 | 57·6 | 53·8 | 29·5 | 33·2 | 29·2 | 34·2 | 45·5 | 24·6 | 14·9 | 37·4 |
| LN | 10·2 | 9·6 | 9·6 | 10·1 | 7·1 | 9·9 | 12·0 | 13·2 | 10·3 | 11·6 | 9·9 | 6·9 | 11·7 | 9·7 | 9·3 | 10·4 | 10·1 |

* = the seven inbred lines shown in fig. 1.

in natural populations, it is clear that we would have rather little confidence in our ability to make such inferences.

TABLE 4

*Number of plants and line means for siliqua number (SN)*

| | Environment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 15° | | 20° | | 25° | | Line mean | |
| Line | N | S.N. | N | S.N. | N | S.N. | N | SN |
| 1 | 10 | 28·7 | 10 | 23·4 | 10 | 13·3 | 30 | 21·8 |
| +2 | 10 | 24·8 | 9 | 19·3 | 10 | 9·5 | 29 | 17·9 |
| 3 | 10 | 19·5 | 10 | 15·5 | 10 | 9·1 | 30 | 14·7 |
| 4 | 10 | 25·0 | 10 | 15·6 | 10 | 10·6 | 30 | 17·1 |
| 5 | 10 | 16·7 | 10 | 12·6 | 9 | 8·2 | 29 | 12·5 |
| 6 | 10 | 21·1 | 9 | 19·1 | 10 | 12·8 | 29 | 17·7 |
| 7 | 7 | 23·9 | 10 | 17·9 | 10 | 9·4 | 27 | 17·1 |
| +8 | 10 | 28·6 | 10 | 22·5 | 9 | 9·4 | 29 | 20·2 |
| 9 | 10 | 22·0 | 10 | 19·2 | 7 | 9·0 | 27 | 16·7 |
| 10 | 2 | 29·0 | 10 | 18·4 | 10 | 12·0 | 22 | 19·8 |
| +11 | 10 | 17·5 | 10 | 13·5 | 10 | 7·0 | 30 | 12·7 |
| 12 | 10 | 19·1 | 9 | 13·3 | 10 | 9·5 | 29 | 14·0 |
| +13 | 10 | 20·5 | 10 | 12·4 | 10 | 10·1 | 30 | 14·3 |
| 14 | 10 | 21·9 | 10 | 13·2 | 9 | 9·0 | 29 | 14·7 |
| 15 | 10 | 20·4 | 10 | 14·3 | 9 | 7·1 | 29 | 13·9 |
| +16 | 9 | 25·2 | 8 | 18·4 | 10 | 11·4 | 27 | 18·3 |
| 17 | 2 | 27·0 | 8 | 16·9 | 2 | 7·5 | 12 | 17·1 |
| 18 | 9 | 19·4 | 8 | 18·8 | 6 | 17·2 | 23 | 18·5 |
| +20 | 10 | 24·4 | 10 | 16·5 | 9 | 14·0 | 29 | 18·3 |
| 22 | 7 | 23·9 | 3 | 21·0 | 2 | 5·5 | 12 | 16·8 |
| 23 | 10 | 21·1 | 10 | 17·5 | 10 | 9·5 | 30 | 16·0 |
| 24 | 10 | 22·1 | 4 | 19·0 | 4 | 13·0 | 18 | 18·0 |
| 25 | 10 | 24·2 | 9 | 16·8 | 9 | 10·7 | 28 | 17·2 |
| +26 | 10 | 21·0 | 10 | 17·5 | 9 | 15·9 | 29 | 18·1 |
| 27 | 6 | 20·0 | 10 | 11·3 | 10 | 13·7 | 26 | 15·0 |
| 28 | 10 | 18·2 | 10 | 11·8 | 10 | 8·1 | 30 | 12·7 |
| 29 | 10 | 28·3 | 10 | 20·3 | 10 | 15·3 | 30 | 21·3 |
| 33 | 10 | 28·8 | 10 | 20·6 | 10 | 9·1 | 30 | 19·5 |
| 34 | 10 | 33·0 | 10 | 25·6 | 10 | 13·5 | 30 | 24·0 |
| 35 | 9 | 25·2 | 10 | 22·1 | 9 | 13·3 | 28 | 20·2 |
| 37 | 10 | 23·3 | 10 | 16·0 | 10 | 8·8 | 30 | 16·0 |
| 39 | 9 | 18·4 | 10 | 18·5 | 9 | 11·4 | 28 | 16·1 |
| 40 | 10 | 22·8 | 10 | 17·1 | 10 | 9·7 | 30 | 16·5 |
| Environment means | 300 | 23·2 | 307 | 17·5 | 292 | 10·7 | 899 | 17·1 Grand mean |

(ii) *Seed and siliqua number*

The purpose of measuring siliqua number in this experiment is to enable us to assess the adaptive significance, if any, of the variability of the primary characters. We need therefore, before proceeding further, to satisfy ourselves as to the adequacy of this character as a measure of reproductive performance.

The total number of seeds produced by a plant is perhaps a more direct measure of reproductive output, particularly in this species in which the germination percentage of fresh seed is very high. The seed is, however, very small and plants are capable of producing many hundreds of them,

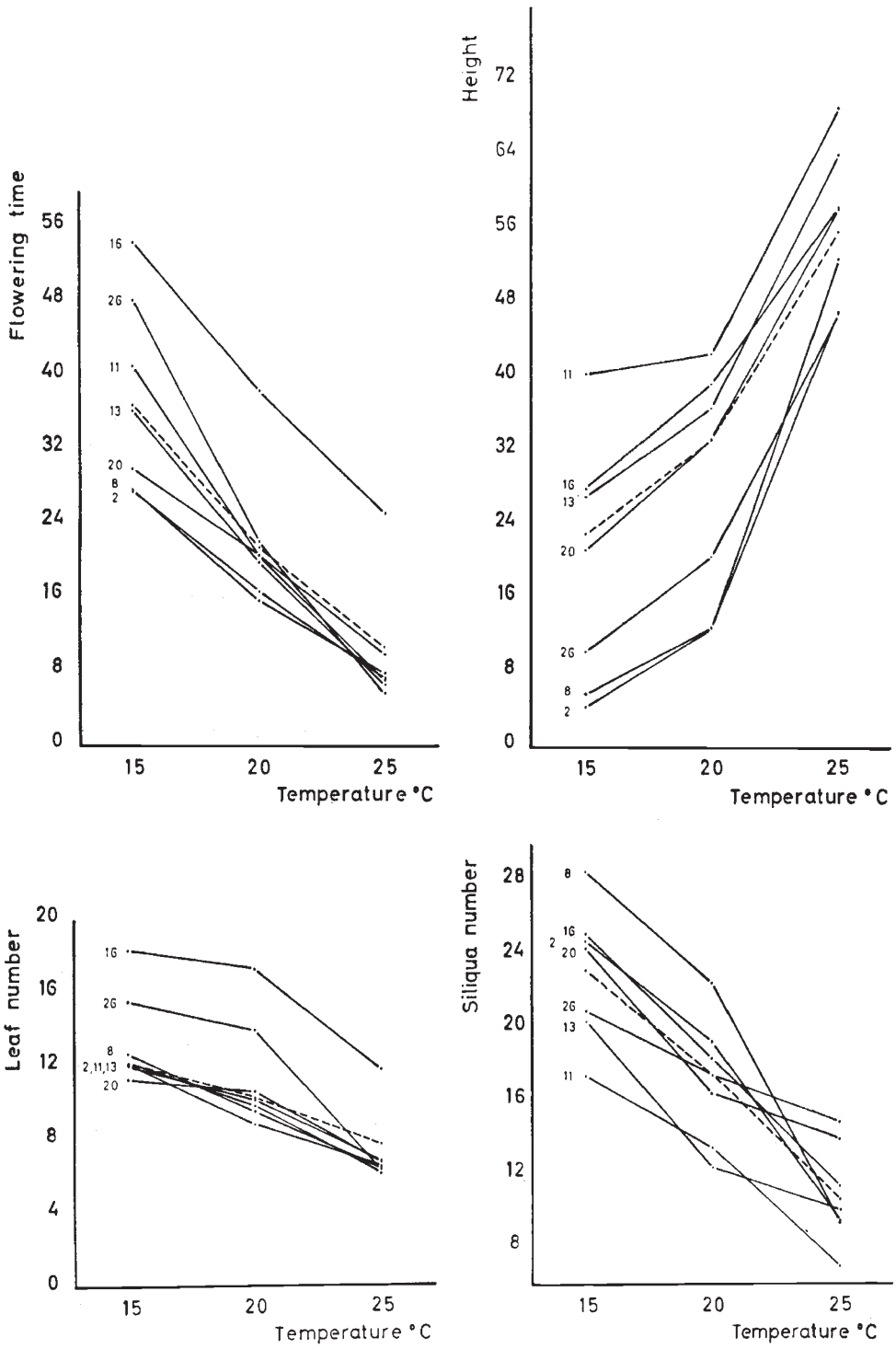


FIG. 1.—Line means and temperature with respect to flowering time, height, leaf number and siliqua number. The lines shown are those indicated in table 2.

even when, as here, they are grown in test-tubes on agar medium. Siliqua number is, therefore, a more convenient measure of reproductive performance. On the other hand, we could seriously underestimate the reproductive output of plants bearing a large number of siliquae if they had a tendency at the same time to produce a large number of *large* siliquae—a property encountered in many other species.

TABLE 5

*Analyses of variance. Entries are mean squares, the significance of which is indicated in the conventional manner*

| Source | d.f. | FT | HT | LN | SN |
|---|---|---|---|---|---|
| Lines (L) | 32 | 2·50*** | 7·62*** | 0·55*** | 0·65*** |
| Environments (E) | 2 | 135·22*** | 138·74*** | 8·62*** | 42·80*** |
| Blocks (B) | 1 | 0·43*** | 0·75* | 0·26*** | 1·26*** |
| L×E | 64 | 0·43*** | 0·99*** | 0·08*** | 0·19*** |
| L×B | 32 | 0·03 | 0·31*** | 0·01 | 0·13** |
| E×B | 2 | 0·04 | 0·23 | 0 | 0·04 |
| L×E×B | 62+ | 0·04*** | 0·21** | 0·03*** | 0·07 |
| Replicates | 1324++ | 0·02 | 0·14 | 0·01 | 0·09 |

\+ = less 2 d.f. because of 2 missing line-block entries.
++ = 703 d.f. in the case of SN.

Ripe siliquae were accordingly harvested daily from five plants in each block and in each cabinet of each of the seven lines mentioned earlier and their mean seed yield determined at the end of the experiment. It turns out that the relationship between mean seed and mean siliqua number is in fact essentially linear (fig. 2). We may therefore proceed with some confidence to regard siliqua number as an adequate measure of reproductive performance in the present circumstances.

(iii) *Joint regression analysis*

The results of the analysis of line means shown earlier leave no doubt that all four characters manifest genotype-environment interaction. We now wish, therefore, to turn to a more detailed analysis of this source of variation and two questions are of particular interest in this respect. Firstly, what is the nature of the response that these lines make to different environments; is it linearly related to the environment or is it otherwise? Secondly, how is this response with regard to the primary characters related to siliqua number?

Now the first question can be answered, of course, by partitioning the Lines x Environments items of the aforegoing analyses of variance into linear and non-linear parts—a procedure which amounts to obtaining the joint regression of line means on temperature. This method, however, is open to the criticism that the centigrade scale on which temperature is measured is physiologically quite arbitrary. A better measure of the environment may be obtained by regressing the line means on to the environmental means, the latter being calculated as the average performance of all lines raised in that environment. In this way, due allowance can be made for the fact that, for example, a shift in temperature from 15° to 20° C. has a less pronounced effect on height, than the equivalent interval on the centigrade scale, from 20° to 25° C. The joint regression analysis of Perkins

and Jinks (1968*a*, *b*) is carried out in this way. The results obtained from the present data using this analysis are shown in table 6.

It is convenient to relax the distinction between temperatures and blocks here, so that the regression analysis concerns six rather than three

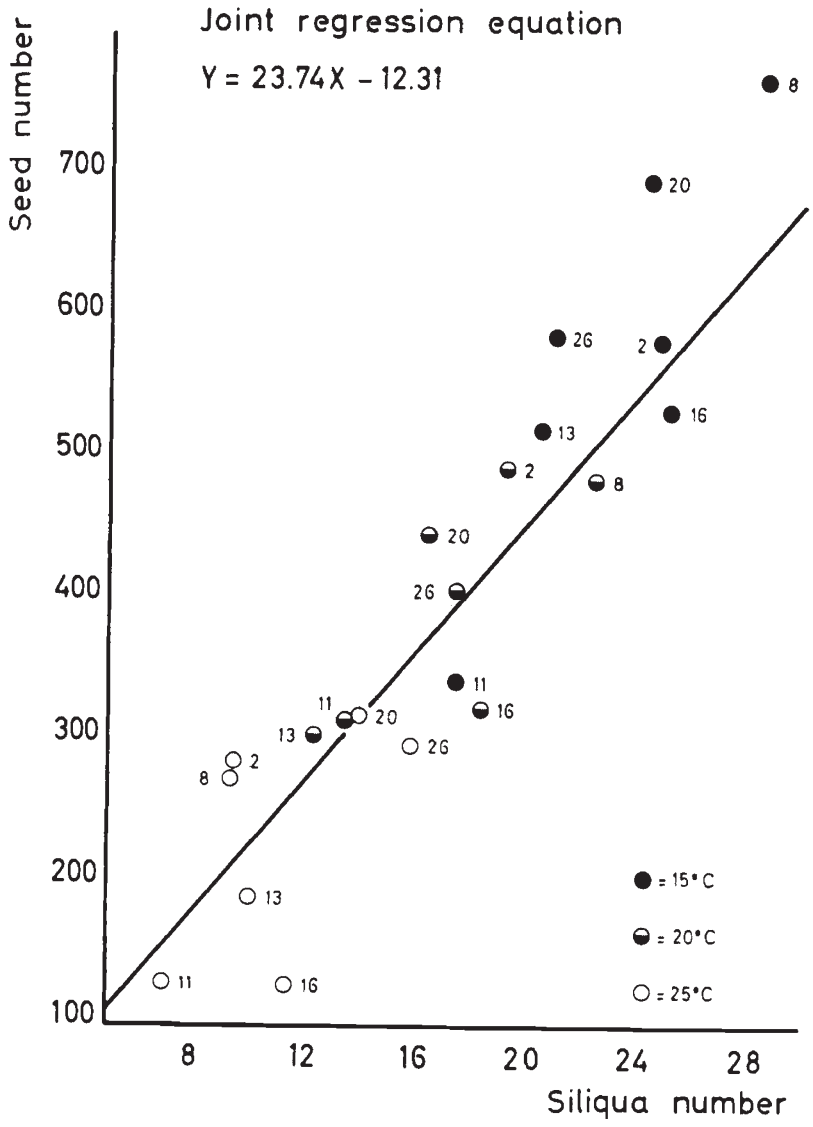


FIG. 2.—Seed and siliqua number with respect to the seven lines.

environments. The Environments item of the present analysis is thus the pool of the Environments, Blocks and E × B items of the previous analysis and in a similar way, the L × E item here is the pool of the remaining interaction items of the analysis of variance. The Lines item, is, of course, identical.

The outcome of this analysis is very clear, for though both the linear and

non-linear items are significant with respect to all four characters, the former is much larger. Indeed, examination of the components of these mean squares shows that linear effects account for 96, 89, 85 and 94 per cent. of the total variation ascribable to genotype-environment interaction with regard to flowering time, height, leaf number and siliqua number respectively. In view of the diverse origins of these lines this outcome is unexpected, for it implies that though their performance changes differentially with the environment, the effect of the latter on the genes determining these characters is essentially similar. Whether this similarity is due to the fact that all the lines are of European origin, or because they have been subject to directional selection, as a consequence of their having been maintained in a laboratory environment for many generations is, at present, far from clear.

TABLE 6

*Joint regression analyses. Entries are mean squares*

| | Source | d.f. | FT | HT | LN | SN |
|---|---|---|---|---|---|---|
| | Lines (L) | 32 | 2·50*** | 7·62*** | 0·55*** | 0·65*** |
| | Environments (E) | 5 | 54·19*** | 55·77*** | 3·50*** | 17·38*** |
| L×E | Heterogeneity between regressions (linear) | 32 | 0·76*** | 1·46*** | 0·12*** | 0·26*** |
| L×E | Remainder (non-linear) | 126+ | 0·05*** | 0·31*** | 0·03*** | 0·10* |
| | Replicates | 1324++ | 0·02 | 0·14 | 0·01 | 0·09 |

Symbols as in previous table.

(iv) *The primary characters and siliqua number*

We can now turn to the second question concerning the nature of the genotype-environment interaction of the primary characters, namely, the question of its relationship with siliqua number.

The predominant linear nature of line performance with respect to the environment, which emerges from the joint regression analysis, suggests a convenient measure of this response. The metric in question is the regression coefficient, $\beta'_i$, of the linear additive model assumed for the purpose of the joint regression analysis to describe an observation on a line mean. This is

$$Y_{ij} = \mu' + d'_i + (1 + \beta'_i)\epsilon'_j + \delta_{ij}$$

(p. 341 of Perkins and Jinks, 1968*a*).

A line with an average response to the environment is expected to have a $\beta'_i$ equal to zero. Similarly, lines with a greater than average or with a less than average response are expected to have $\beta$'s of greater than or less than zero respectively. Thus we can, in this way, characterise the relative response of all lines in the experiment. The relationship of this metric with average siliqua number, a comparison which can be made for each inbred line, thus affords a convenient means of deciding what kind of interaction is likely to be adaptive in the context of this experiment.

We have chosen to estimate the quantity $(1+\beta'_i)$ rather than the regression coefficient as such; the average value of these quantities becomes, in consequence, one rather than zero. Fig. 3 shows the relationship between this relative response metric and siliqua number for each line and for each of the three primary characters.

The interpretation of these diagrams is a straightforward matter. Firstly, since all relationships are necessarily relative, we are chiefly concerned with the distribution of the points about the average values of the ordinate and abscissa. These average values are indicated by the broken

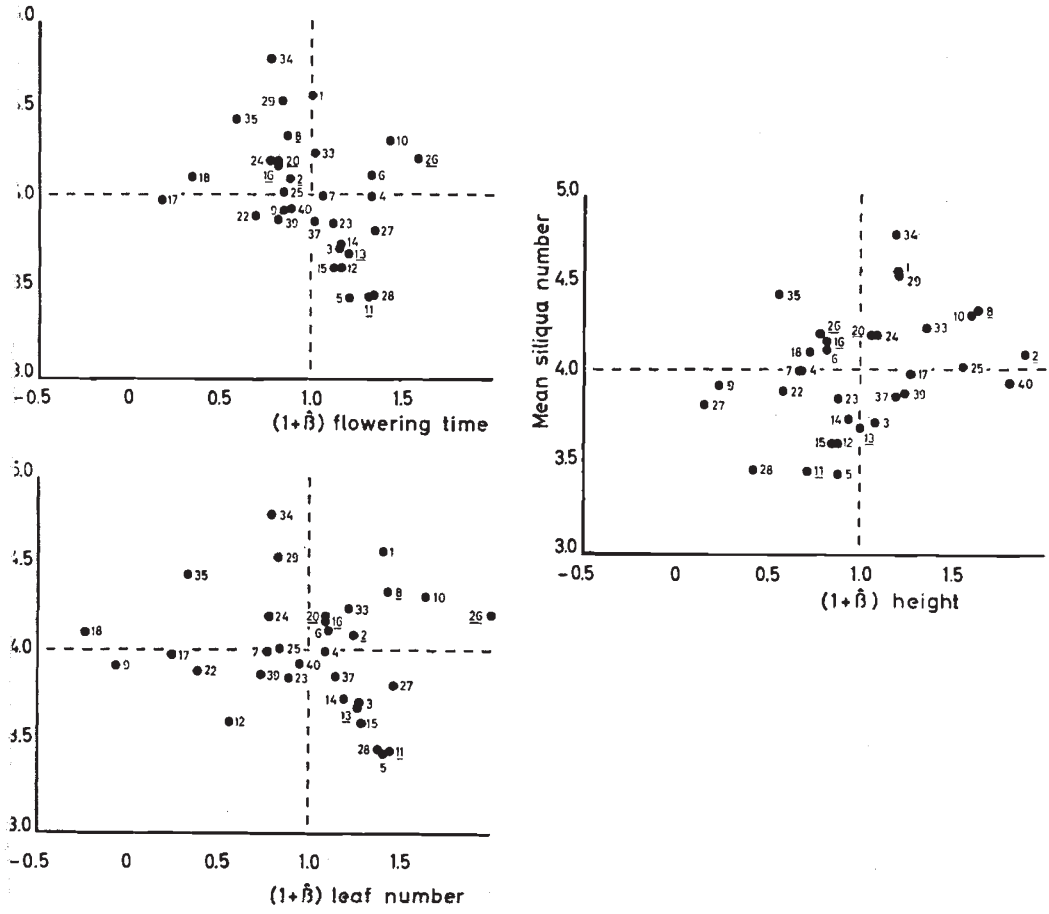


FIG. 3.—Mean siliqua number and the response metric $(1+\beta_i')$ for flowering time, height and siliqua number.

lines of the diagram. Secondly, the imposition of these average value axes divides the diagram into four parts, which correspond to the four basic types of developmental regulation shown earlier in table 1; the latter, therefore, can be regarded as a " mask " for the purpose of interpretation.

This procedure can be illustrated with reference to the flowering-time diagram. The points tend in this diagram to lie about a line running from the top left to the bottom right quarter, a tendency which turns out to be just significant. In other words, those lines whose flowering time changes less than the average with temperature (*i.e.* $(1+\beta_i')<1$) appear to produce more siliquae than those lines whose flowering time changes more than average (*i.e.* $(1+\beta_i')<1$). The conclusion here is thus clear. On average,

the fittest lines are those whose flowering time is well buffered or which are developmentally stable.

Not all the points in this diagram, however, lie within the top left-hand and bottom right-hand quarters. For example, while the points of six of the seven lines mentioned earlier (fig. 1) fall within the main cluster, that of the seventh (line 26) lies well outside in the top right-hand quarter of the diagram. Indeed, this line is of considerable interest since, while it is the most reactive of all the lines in the experiment, it produces a higher than average number of siliquae. The development of flowering time in this line, therefore, appears to be able to respond in an adaptive manner to different environments; that is, the line displays developmental flexibility. Although line 10 appears to be similar to line 26 in this respect, we lack their counterpart, a line whose development is inflexible and whose point would be expected to fall in the bottom left-hand quarter of the diagram.

These lines apart, the predominant mode of developmental regulation here appears to be such that stabilisation is advantageous. In general, we have no expectation that a set of unrelated inbred lines will display any consistent pattern of developmental regulation, although a set of common origin might well do so. That it is possible, therefore, to fit a straight line to these data appears to be quite fortuitous, irrespective of whether the relationship between siliqua number and the response metric is direct or via a correlation between these and mean flowering time. In point of fact, mean siliqua number is not related to mean flowering time, nor is the latter related to the response metric in these data, so there is no question of an indirect relationship between the former and the latter here.

Turning now to the height diagram, it is clear that the predominant trend here is opposite to that of flowering time, for the points are clustered around a line running from the top right-hand to the bottom left-hand quarters. Though this trend is not quite significant, those lines with the highest reproductive output display a relatively high degree of developmental flexibility with respect to height. Once again, however, not all lines conform to this general pattern; line 35, for example, is one whose development of the character height appears to be quite strongly stabilised.

The development of leaf number is apparently not regulated in any particular manner. Since in terms of their average phenotype, leaf number and flowering time are quite highly and positively correlated, this outcome is unexpected. The developmental phenotypes of these characters are clearly not at all related.

One further point concerning the interpretation of these diagrams deserves mention. We have argued that those lines whose points fall to the left of the ordinate at $(1+\beta_i') = 1$, display a lower than average response to environmental variation. This agreement holds only if the condition $0 < (1+\beta_i') < 1$ is satisfied. We notice, for instance, that in the leaf number diagram, the points of lines 18 and 9 lie to the left of $(1+\beta_i') = 0$. Since, by definition, the expected value of the response metric is unity, lines 18 and 9 are in fact *more* reactive than one which yields $(1+\beta_i') = 0$. In short, it is the absolute value of this quantity which is of importance. In the present case, this causes no difficulty, for line 9 responds less than, and line 18 to about the same extent as, line 17, which is otherwise the least reactive line of the experiment.

## 4. Discussion

The results from this investigation leave little doubt that there are considerable genetic differences between the inbred lines in respect of their developmental phenotype. These lines also differ with respect to their average phenotype. We may ask, therefore, whether there is any relationship between these two aspects of the total phenotype of a character which would cause us to suggest that both are determined by the same genotype.

This question is easily answered, for the primary characters fall into two groups in this respect. In the first group, which includes flowering time and leaf number, there is no relationship between the average phenotype of a line and its developmental phenotype. Each must, therefore, be determined by different genotypes, a state of affairs which, where this is desirable, would permit the adjustment of each phenotype by natural selection independently of the other. Height, on the other hand, is different, for it turns out that there is a highly significant negative correlation between the mean expression and the response metric of this character ($r = -0{\cdot}696$, $P < 0{\cdot}001$); that is to say, short plants vary more with the environment than tall plants. Thus, on our previous argument concerning the developmental regulation of this character, the development of height in short plants is more flexible than that in tall plants; that is, response in the former is of the adaptive type.

The correlation between average and developmental phenotype here raises the question of whether this is due to the pleiotropic effects of the same genotype or whether, alternatively, the correlation is due to linkage between two otherwise independent genotypes. In view of the origins of these inbred lines, the latter is unlikely. The development of this character therefore cannot, it appears, be adjusted independently of the average phenotype by natural selection, unless, of course, the observed association is due to chance alone.

## 5. Summary

1. In principle, the evolutionary role of genotype-environment interaction in the population genetics of a species may take one or other of two mutually exclusive forms; the expression of a metrical character may be buffered against the environment (the character is *developmentally stable*) or may vary in an adaptive manner with the environment (the character is *developmentally flexible*).

2. The relationship between three primary characters, flowering time, height and basal leaf number, on the one hand, and the fitness character, siliqua number on the other, has been examined in thirty-three inbred lines of *Arabidopsis thaliana* raised at 15°, 20° and 25° C. in controlled environments, with this point of view in mind.

3. Though the genotype-environment interaction which all four characters display is small relative to the total phenotypic variance, it is large relative to the genetic variance of the character.

4. The relationship between the performance of a line and the environment in which it is raised is a simple one in that this relationship is essentially linear for both the primary and the fitness characters.

5. The relationship between flowering time and siliqua number suggests

that the development of the former is generally well-buffered in those lines with a higher than average siliqua number; that is, the development of this character is better stabilised in these lines than in others.

6. Those lines whose height changes least with the environment, on the other hand, produce in general a lower than average number of siliquae; thus the development of this character in lines with a superior reproductive performance, appears to be able to respond in a flexible or adaptive manner to variation of the environment.

7. Not all lines with respect to either flowering time or height accord with these general conclusions, however, and there appears to be no predominant mode with regard to the development of the third primary character, leaf number.

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