Theoretical and Applied Genetics 41, 285–291 (1971)

Simulation of Quantitative Characters by Genes with Biochemically Definable Action

II. The material

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Summary. An experimental system has been developed which allows the application of the mathematical model given in Part I of this series. It consists of all possible genotypic combinations of four loci involved in the modification of the anthocyanin molecule structure by oxydation, glycosidation, and acylation in the annual cruciferous garden plant *Matthiola incana*. These genes are varied against a background which is nearly isogenic with respect to the concentration of flower pigments. The qualitative actions and interactions of the genes and the composition and isogenisation of the genetic background are described.

As a simulated quantitative character the total concentration of anthocyanins per petal fresh weight is used and measured in a reading spectrophotometer.

The genetic structure of the material is a prerequisite for the analysis by means of the earlier given model, and the knowledge of the qualitative actions and interactions of the involved genes may be useful in the interpretation of the estimations of the parameters and possibly facilitate an improvement of the model.

Introduction

In a preceeding publication (Seyffert, 1966) the project was explained in which a metric character was simulated by means of genes, the effect of which can be defined qualitatively as well as measured quantitatively. At the same time a simple model was described that allows the estimation of additive, dominant, and epistatic contributions of several genes and gene combinations, both of which were varied in the experiment. In its disposition the model corresponds deliberately with those up to now in the genetics of quantitative characters, i.e. it is based on a linear combination of all components involved in the expression of the phenotype. It differs from other models by allowing the estimation of not only the total effect of all genes concerned as part of the variance of a population but also the individual contribution of each single allele and the contribution of the genetic background at the measured value of the respective genotype. Presuppositions for the application of this model are

1) all realizable combinations of the gene pairs to be examined, because otherwise losses in information are inevitable and

2) a largely isogenic genetic background in order to avoid an adulteration of the estimation of parameters.

Moreover, the experimental material that is to be used for the simulation of metric traits must meet the following minimum requirements, apart from the above mentioned:

3) disomic inheritance,

4) no sex limited or sex linked inheritance,

5) no genes with lethal effects with regard to the character to be measured.

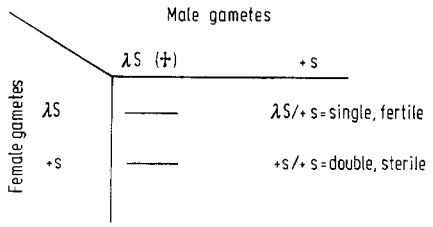
On the other hand, neither linkage nor interactions between genotype and environment are disturbing, because in the first case they are eliminated by the design of experiments, and in the second case they can be estimated directly.

This paper deals with the structure and description of the experimental material. It is intended that in further publications full particulars will be given on the application of the model, the comparison of the results obtained in this way with those obtained by means of other methods, and on the variation of the model by changing the assumptions and/or presuppositions.

Material

For the intended examination the annual cruciferous garden plant *Matthiola incana* R. Br., and in particular the character "total content of anthocyanin pigments per petal fresh weight" seems to be especially suited for the following reasons: The cultivated stock is a mainly inbreeding plant whose inflorescence — genotypically controlled — is branched to varying degrees and has many successively opening single or double flowers. Therefore during flowering time from July to August flowers of various stages at each plant are available continuously. For the examination of the flower pigments the double flowers are well suited because of their large number of petals, their longer flowering and the lack of

sexual organs, whereas the single flowering plants may be used for seed production and further propagation. Detailed examinations exist by Kappert (1935, 1937) on the genetics of the doubleness of flowers. He has proved that the typical eversporting of the cultivated stock is to be traced back to a balanced system of a gonic lethal factor (λ) linked closely with the recessive gene for doubleness. Apart from rare recombinants, all inbreeding and outbreeding progenies of the cultivated stock consist half and half of single fertile and double sterile types.



The cultivation of the material begins in the greenhouse at 10°—15 °C after sowing in February/March. Late in April/beginning of May the plants are planted with a space of 15—20 cm in the open field. Flowering begins in the middle of June, the main flowering time is in July and August. Flowers opening later frequently show virus infection (mosaic, "breaking of the flower colour"). They are then no longer suited for the planned analysis but are for the seed propagation because the turnip-mosaic-virus is not transferable this way.

Matthiola's inbreeding and outbreeding technique is simple. The low crossfertilization rate of 10^{-3} to 10^{-2} and the use of marker genes allow the plants to flower unprotected without dangering the reliability of the selfing or the cross. The seeds ripen relatively late, frequently at the time of early frost in October or November. The propagation rate is sufficient for the present experiment. One pod contains after inbreeding approximately 20 to 60 seeds and one plant according to the degree of branching approximately 1 to 3 time 10^3. The amount of seeds after outbreeding is lower.

The choice of the character "total content of anthocyanin pigments per petal fresh weight" takes place from two points of view:

1) Each gene used for simulation should be defined as clearly as possible, i.e., it should have a qualitative action and, if possible, also an interallelic interaction with other genes participating in the simulation. This is more likely if one works with genes participating in the expression or modification of the same biosynthesis chain and controlling as end product a molecule which is simple to examine and has a structure that can be varied sufficiently.

2) It should be guaranteed that the qualitative changes lead at the same time to measurable differences in the concentration of end products, and that the genetic variation of a number of model genes will be expressed in a continuous variation of the measured value when the total concentration of all qualitatively changed components is measured. Former examinations of the inheritance of flower colour of stock (Jungfer 1957, Seyffert 1960) have shown that the points of view mentioned under 1) are correct for the genes contributing to the modification of the anthocyanins. In addition unpublished papers have shown that the choice of the character with regard to the second point of view is also justified, because a measurement of the total concentration of the pigments in segregating progenies has revealed a continuous variation of the expression of the trait. In the following the genetic characteristics of the material used and its composition with regard to the intended experiment will be described.

Presuppositions for the occurrence of anthocyanin pigments in the petals of *Matthiola incana* is a workable flavonoid-biosynthesis pathway. Already in 1949 Kappert proved that this pathway can be interrupted in three different places by independently segregating and complementarily acting genes. The existence of a recessive allele at the *e*-, *g*- or *f*-locus is sufficient to stop the synthesis of the anthocyanins completely, i. e. to cause white or yellowish-white flowering forms. Double heterozygote ee^+gg^+, ee^+ff^+, or gg^+ff^+ types lead therefore after selfing to a 9:7 segregation of coloured to white flowering, and triple heterozygote types $ee^+gg^+ff^+$ to a 27:37 segregation. The single recessive white flowering mutants are not distinguishable externally. According to preliminary unpublished (Thomas) and not yet confirmed examinations of Willweber (1968) they show in their pattern of flavonoids in part a complementary distribution of potential anthocyanin precursors.

Effects of the anthocyanin modifiers

The final expression of the anthocyanin type is decided by a number of modifying genes that cause different substitutions. Up to now four different loci are known that modify subsequent to the synthesis of anthocyanins. As their effect has already been described elsewhere (Seyffert 1960, 1962b, Harborne 1962) a brief description will be sufficient.

The pair of alleles b/b^+ is responsible for the variation of the anthocyanidin type. In the presence of the recessive allele pelargonidin derivatives will be formed exclusively; in the presence of the dominant allele cyanidin derivatives predominate. The wild allele proves to be qualitatively completely dominant, because homo- and heterozygote genotypes do not differ either phenotypically or in their chromatographically examined pigment pattern. Yet both genotypes show not only cyanidins but also small quantities of pelargonidin derivatives. These pelargonidins undergo by means of interaction with further modifying genes described in the following slight but not further examined variations. Qualitatively re-

cognizable interactions with the other loci (*l*, *u*, *v*) have not yet been observed. The phenotypical manifestation of the recessive allele *b* consists in a carmine pigmentation. If the other loci are occupied by their respective wild alleles, the dominant allele b^+ changes the colour to violet. The dominant wave length (λ_D) of the spectrum of remission in the CIE-system accordingly changes for the complementary colour from $\lambda_D = 494$ nm for carmine (*bb* ++ ++ ++ ++)* to $\lambda_D = 520$ nm for violet (++ ++ ++ ++ ++), (Seyffert 1963).

The pair of alleles l/l^+ is responsible for a glucosidation of anthocyanins. Anthocyanidins are not found natively in the cell. They are always glycosidated, i. e. present as anthocyanins. *Matthiola incana* has in double recessive genotypes (..*ll uu*..) two types of glycosides side by side: 3-monoglucoside and 3-bioside, whereby the disaccharide has been described by Harborne (1963) as xylosylglucose ("sambubiose"). In case the recessive allele (*l*) is replaced by its completely dominant partner (l^+) the pattern of glycosides changes in such a way that 3-biosides disappear completely and instead a new type of glycoside appears which can be identified as 3-xylosylgluco-5-glucoside. A further new type of glycoside may be identified as 3,5-diglucoside, but it appears only in slight concentrations. It seems that the 3-monosides initially existing have been reduced by approximately the amount of the newly appearing 3,5-dimonosides.

The effect of the l^+-allele consists in a glucosidation of the 5-position of the anthocyanins in which the 3-biosides have apparently been converted totally, the 3-monosides only partially. The phenotypic change can best be described as change from slaty (*ll*) to bright colour expression (l^+l^+). The spectrum of remission of the complementary colour shifts only slightly from $\lambda_D = 500$ nm (++ *ll uu* ++) to $\lambda_D = 493$ nm (++ ++ *uu* ++), and from $\lambda_D = 528$ nm (++ *ll* ++ ++) to $\lambda_D = 520$ nm (++ ++ ++ ++ ++).

The pair of alleles u/u^+ is marked by a multiple pleiotropy which gives rise to the conjecture that it is a regulating element which controls a number of structural genes. The pleiotropy manifests itself in a variable influence on the pattern of glycosidation and on the pattern of acylation, whereby the different functions are apparently independent of each other (Seyffert 1962b). With regard to the glycosidation the allele u^+ shows an interesting double function. Starting from the double recessive genotype (.. *ll uu* ..) that contains 3-monoglucoside and 3-xylosylglucoside, after introduction of the u^+-allele 3-xylosylglucoside is found instead of 3-monoside. The structure of the 3-xylosylglucoside however remains unchanged. That means that the 3-biosides as substrate are apparently not used for this kind of conversion, whereas the 3-monosides are changed to triglycosides by substituting one molecule of glucose in the 5- and xylose in the 2-position of the glucose in the 3-position of the anthocyanins. The u^+-allele thus can partially replace the effect of the l^+-allele with regard to the substitution of the 5-position, whereby the variable affinity to the substrate must be considered:

l^+ converts 3-biosides completely and 3-monosides only partially,

u^+ does not convert 3-biosides at all but 3-monosides completely.

Not only the glycosidation but also the acylation pattern of the anthocyanins is changed by u^+. The double recessive genotype (.. .. *uu vv*) contains only unacylated anthocyanins. In addition to the above described change of the glycosidic structure the introduction of the u^+-allele causes the esterification of the biosides and triosides with cinnamic acid derivatives. An approximately equally large part of the bio- and triosides with 4-oxycinnamic acid (p-coumaric acid), 3,4-dioxycinnamic acid (caffeic acid) and 4-oxy-3-methoxy-cinnamic acid (ferulic acid) is combined with the anthocyanin probably over a free OH-group of the xylose. An exact localization of the substitution is not easily possible because of the acyl migration (Lemke, unpublished) observed by the extraction of the pigments. Starting from the double recessive genotype (.. ++ *uu vv*) the change by replacement of the *u*-allele by the u^+-allele leads without exception to the formation of triglycosides that are estered with p-coumaric-, caffeic- and ferulic acid. The phenotypic expression, compared to the wild type (violet), can be described by an umbra or brown-violet colouring observed in the presence of the recessive *u*-allele. The spectrum of remission of the complementary colour changes from $\lambda_D = 520$ nm for the wild type to $\lambda_D = 493$ nm for brown-violet (++ ++ *uu* ++).

The pair of alleles v/v^+ is exclusively responsible for the acylation of anthocyanins. Contrary to the above described locus it controls a somewhat aberrant pattern of cinnamic acids that coincides only partially with that of the u^+-allele. The comparison between a double recessive (.. .. *uu vv*) genotype and a single recessive of the type (.. .. *uu* ++) shows that the dominant v^+-allele causes the esterification with 4-oxy-3-methoxy-, 3,4-dioxy- and 4-oxy-3,5-dimethoxy cinnamic acid (sinapic acid). p-coumaric acid as the acyl component is not observed. A further characteristic appears with triglycosides (genotype .. ++ *uu* ++) which is apparently also a multiple esterification with two different cinnamic acids, because after alkaline hydrolysis of purified acylated triglycosides in addition to caffeic-acid, sinapic- or

* The marking of the genotype is made for all four loci in the sequence *bb ll uu vv*. The manner of writing (*bb* ++ ++ ++ ++) designates the monogenic recessive mutant *bb*, and (.. *ll uu* ..) a double recessive type for which with regard to its meaning it is unimportant in which way the loci *b* and *v* are occupied.

ferulic acid can be obtained. It is not clear whether the acylation of the anthocyanins using the three mentioned cinnamic acids occurs randomly, and thus all three possible diacylated triglycosides develop in addition to the 3-monoacylated, because the hitherto obtained chromatographic separations do not allow a definite answer. The phenotypic change, starting from the wild type (violet), can be described as a change to lilac (++ ++ ++ *vv*); the dominating wave length of the complementary colour changes correspondingly from $\lambda_D = 520$ nm to $\lambda_D = 529$ nm.

Interactions between anthocyanin modifying genes

The description of the effect of the four modifying loci shows that non-allelic genes control in part similar functions and can thus be substituted for one another, but their effects are partially or completely dependent on the state of another locus. In particular this case arises if one + allele at one locus provides the qualitative prerequisite for the effect of the + allele at the other locus.

Characteristic for the first mentioned type is not only the glucosidation of the 5-position of the anthocyanin, which, depending on the respective substrate, can occur by l^+ as well as by u^+, but also the acylation with 3,4-dioxy- and with 3-methoxy-4-oxycinnamic acid controlled by the genes u^+ and v^+.

The genetic analysis of the F_2 shows, as is typical for such cases, the 15:1-segregation of a duplicate gene action (Seyffert 1962b, p. 22, 24).

The second kind of interaction manifests itself genetically either (in the absence of u^+) in a 9:7- or (in the presence of u^+) in a 13:3-segregation. Both cases indicate that an acylation with 3,4-dioxycinnamic acid by means of gene v^+ requires a glucosidation caused by gene l^+ (Seyffert 1962b, p. 24). The modification of a 9:7-segregation to a 13:3-segregation by introducing the u^+-allele is based on the duplicate gene action between l^+ and u^+.

Thus a large number of genetically traceable interactions exists between the loci *l*, *u* and *v*, whereas the locus *b* has shown no perceptible interactions with the three other loci because the pelargonidin- respectively cyanidin-derivatives controlled by this locus show basically the same pigment pattern with regard to the variation of the three other loci. The biochemical

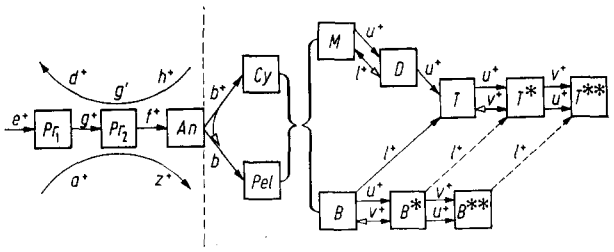


Fig. 1. Scheme of the genetic structure of the experimental system. Genes in the isogenic background are left of the broken line, genes varied in the experiment to the right. Explanation of symbols: *Pr* = precursor, *An* = anthocyanidine, *Pel* = pelargonidine, *Cy* = cyanidine, *M* = 3-monoglucoside, *B* = 3-xyloglucoside, *D* = 3,5-diglucoside, *T* = 3-xylo-gluco-5-glucoside, * = monoacylated, ** = diacylated; two arrow heads = equilibrium with a shift in the direction of the solid arrow head. The genotype of the genetic background is $e^+ e^+ g^+ g^+ f^+ f^+ a^+ a^+ z^+ z^+ hh$ (*dd*)

principles of interaction have not been examined, but their analysis could give valuable information for the genetics of quantitative characters as well. The scheme shown in Figure 1 results when all hitherto known data on the gene action and interaction of the above mentioned anthocyanin modifiers, including the unpublished results of Lemke are combined and completed by information on genes (p. 289) that accelerate or inhibit the anthocyanin biosynthesis. In this figure the loci (left) that have not been varied in the experiment and are kept isogenic in the genetic background are separated by the vertical dividing line from the loci (right) varied in all genotypic combinations. Arrows that point in both directions indicate equilibrium with a shift in the direction of the solid arrowhead. This scheme can be used as a working hypothesis, whereby with quantitative genetic examinations attention must be paid especially to the analogy between quantitative interaction parameters to be estimated and the qualitative connections shown here.

In this figure only a general marking of singly (*) or doubly (**) acylated anthocyanins was made because a representation of the total amount of possible and simultaneously present components of the pigment pattern leads to a multidimensional and thus unclear description. As is shown in the supplementary Table 1, up to 11 main components in different concentrations appear side by side, whereby

Table 1. *Schematic survey of the anthocyanin content dependent on the loci l, u and v*

(*l*)	*u*	*u*+
v	M: —	B: C, K, F
	B: —	T: C, K, F
v+	M :—	B: k, F, S, CF, FS
	B: —, F, S	T: k, F, S, KF, KS,FS

(*l*+)	*u*	*u*+
v	M: —	
	D: —	
	T: —	T: C, K, F
v+	M: —	T: k, F, s, ck, CF, CS
	D: —	KF, KS, FS
	T: —, kf, KS, FS	

Abbreviations: M = 3-monoglucoside, B = 3-xylosylglucoside, D = 3,5-dimonoglucoside, T = 3-xylosylgluco-5-glucoside, :— = non acylated anthocyanins, :C = acylated with p-coumaric acid, :K = acylated with caffeic acid, :F = acylated with ferulic acid, :S = acylated with sinapic acid, use of small letters = slight concentration

anthocyanins in low concentrations have not been taken into consideration because of the difficulty of separating and identifying them chromatographically.

Arrangement of the gene combinations and isogenisation of the genetic background

The material previously described (Seyffert, 1960) consists of a number of separate lines that already existed in all $2^3 = 8$ possible combinations with regard to the loci *l*, *u* and *v* and against the background b^+b^+, whereas the corresponding combinations in connection with the genotype *bb* had in part not been identified. By crossing of suited parental lines, visual and chromatographic evaluation of the F_1- and F_2-progenies of all 16 homozygote combinations possible with four loci with two alleles each were shown and numbered as seen in Table 2. At the same time an attempt was made to make the genetic

Table 2. *Geno- and phenotypes of 16 homozygote combinations of the loci b, l, u and v including one not completely isogenic dilution factor in the genetic background*

Phenotype	Symbol	Genotype	Dilution factor* 1965	1968	λ max
brown carmine	0000	*bb ll uu vv*	++	++	507
brown carmine	0002	*bb ll uu* ++	*dd*	*dd*	508
dull pink	0020	*bb ll* ++ *vv*	*dd*	*dd*	510
dull carmine	0022	*bb ll* ++ ++	*dd*	*dd*	511
brown pink	0200	*bb* ++ *uu vv*	++	++	509
brown carmine	0202	*bb* ++ *uu* ++	++	*dd*	509
bright pink	0220	*bb* ++ ++ *vv*	*dd*	*dd*	509
bright carmine	0222	*bb* ++ ++ ++	*dd*.	*dd*	512
brown violet	2000	++ *ll uu vv*	++	*dd*	527
brown violet	2002	++ *ll uu* ++	++	*dd*	527
dull lilac	2020	++ *ll* ++ *vv*	++	*dd*	528
dull violet	2022	++ *ll* ++ ++	++	*dd*	528
brown lilac	2200	++ ++ *uu vv*	++	*dd*	526
brown	2202	++ ++ *uu* ++	++	*dd*	526
bright lilac	2220	++ ++ ++ *vv*	*dd*	*dd*	526
bright violet	2222	++++++++	*dd*	*dd*	527

* The genotype of the dilution factor is shown in Table 3.

background of these 16 lines as isogenic as possible in order to ascribe the variation of the measured values of the phenotypes to the experimentally varied loci alone. Since the large number of parental lines makes a total isogenisation impossible, the process was limited to such loci which influence the intensity of the flower pigmentation in a perceptible way. By continuous control of the F_2-families of diallelic crosses of 16 parental lines it should be achieved that

1) residual variations in the genetic background can be controlled and,
2) by selection from segregation F_2- and/or F_3-progenies suitable non segregating and isogenic types with a maximum intensity of pigments can be obtained.

Investigations made by Jungfer in 1957 had shown two loci that apparently influence the quantities of the formed pigments and compensate mutually. Against the background of the pigment quality caused by the above mentioned loci the allele a^+ intensifies and the allele h^+ dilutes the flower colour Double dominant $h^+.a^+$. genotypes as well as double recessive *hhaa* genotypes show a medium colour intensity and can by no means be differentiated except by the reaction to virus infection (Kruckelmann and Seyffert, 1970). The phenotype $h^+.aa$ is very light, the phenotype hha^+. on the contrary deeply coloured. From the beginning the present material was isogenic for *hh* a^+a^+. Moreover, when the gene z^+ is isogenic, it leads to a maximal rate of synthesis, whereas when recessive it reduces in a way similar to *g'*, a multiple allele of g^+ and *g*, the speed of the biosynthesis of the anthocyanins and results in a drastic reduction of pigmentation in younger petals (Seyffert, 1962a). Unpublished experiments from the years 1960—1964 showed, however, that there exist, independent of these diluting factors designated as "strongly acting", a number of further loci with slight and often hardly noticeably differences in intensity. Since the expression of the differences apparently shows an interaction with the pigment pattern, an exact formal genetic analysis in progenies in which the anthocyanin modifiers *b*, *l*, *u* and *v* also segregate is rendered difficult. However, from the careful examination of intensity differences between qualitatively equal phenotypes of F_2-progenies it is possible to draw a conclusion as to the genotype of the respective parental line (Table 3). In 1967a test was carried out that identifies the genotype of the parents used in 1965 for crossing, while the test carried out in 1970 shows the progress in the isogenisation of the material until 1968. The genotypes of the parents are already specified in Table 2, column 4 and 5. The very slight differences in light intensity in some F_2-progenies (symbol "*s*" in Table 3) can possibly be ascribed to a pair of alleles acting more weakly than d^+/d. Where in Table 3 a question mark is used as a symbol, uniformity was designated as uncertain because of the difficulties of an exact comparison between qualitatively differentiated pigment types. All these progenies, however, probably belong to the group of intensely coloured flowers.

The test made in 1967 shows that the phenotypic values measured in 1966 (and also in 1965) cannot be compared in every combination without considering the influence of the genetic background. On the contrary a grouping is necessary in which the d^+- or the *d*-allele is common to all parents used in the diallele. This results in the limitation that the data obtained in these two years can only be examined in small groups of four parents each, i. e. in a bifactorial system, whereas for the values measured from 1969 on three trifactorial 8×8 systems can be selected. Thus the loci *l*, *u* and *v* can be varied in all

Table 3. *Evaluation of diallelic F_2-progenies, test on isogeneity of dilution factors in 1967 and in 1970. Data from 1967 at upper right, from 1970 lower left*

Explanation of the symbols: – = F_2 uniform, slightly diluted; *s* = very slight differences in F_2, × = F_2 segregating, 3 diluted:1 deep, ? = no complete uniformity for deep, + = F_2 uniform, deeply coloured.

In the main diagonal from upper left to lower right the phenotype of parental lines obtained by means of the dialleles is symbolized. The first symbol refers to the diallele made in 1970 and the second to that made in 1967. The results of reciprocal crosses are combined and the entries for each diallele are given below left and above right respectively.

1967

Phenotype / Parental Lines	Parental Lines	0000	0002	0020	0022	0200	0202	0220	0222	2000	2002	2020	2022	2200	2202	2220	2222
	0000	--		×	×	-	×	×		-	-	-	-		-	×	×
	0002	×	++	+	+	×		+	+	×	×	×	×	×	×	+	+
	0020	×	+	++	+	×	×		+	×	×		×	×	×	+	
	0022	×	+	+	++	×	×	+	+	×	×	×	×	×	×	+	+
	0200	?	?	s		--	-	×	×	-	-	-	-	-	-	×	×
	0202	×	+	+	+	×	+-	×	×	-	-	-	-	-	-		×
1	0220	×	+	+	+	s	+	++	+	×	×			×	×	+	+
9	0222	×	+	+	+	×	+	+	++	×	×	×	×	×	×	+	+
7	2000	×	?	?	+	?	?	?	s	+-	-	-	-	-	-	×	×
0	2002	×			+	?	+	+	+		+-		-	-	-		×
	2020	×	+		+	s	+	+	+	?	+	+-			-	×	×
	2022		+	+	+	×	s	+	+	+	+	+	+-	-	-	×	×
	2200	×	?	?	+	×	?	+	s	?		s	s	+-	-	×	×
	2202	×	+	+	+	?	s	+	+	s	+	+	+	+	+-	×	×
	2220	×	+	+	+	×	+	+		+	+	+	+	s		+-	+
	2222	×	+	+	+	s	+	+	+	s	+		+	+	+	+	++

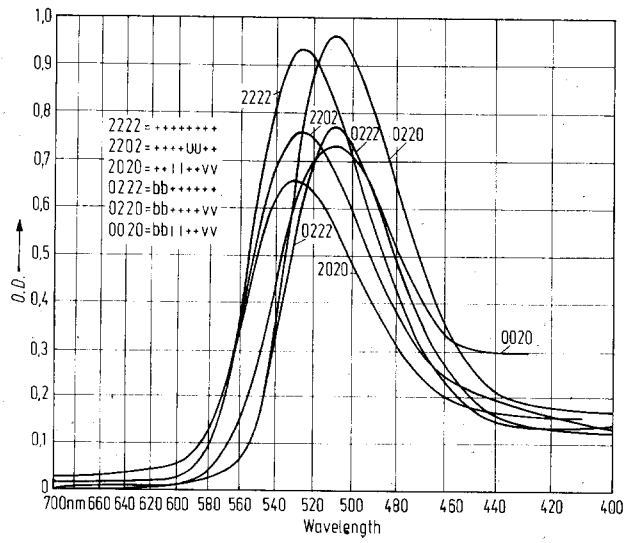


Fig. 2. Graph of the extinction values (O.D.) on the ordinate of six selected genotypes, plotted against the wave-length (abscissa), showing the diversity of the maxima

27 genotypic combinations against the constant background b^+b^+dd; the loci *b*, *l* and *v* against the background u^+u^+dd; and the loci *b*, *l* and *u* against the background v^+v^+dd.

Quantitative measurement of the anthocyanin pigments

There are different ways to measure the total content of the anthocyanins. If in a short time a large number of different types is to be measured at the same time and a high accuracy of measurements is to be obtained, spectrophotometric examinations of extracts of fresh flowers must be considered first. In the present material qualitatively modified pigment patterns were evaluated quantitatively. As the spectrum of different genotypes (Fig. 2) and the last column of Table 2 show, the maxima of the optical density are not identical. Therefore the extinction cannot be measured by means of a constant wavelength produced by filter or lattice. Instead the aim was to integrate the area over the total measuring range and use this as measure for the concentration. Opposed to this is on the one hand the time spent on the measurement over the total spectrum and on the other hand the observation of Birkofer and Kaiser (personal communication) that during the measurement in the UV area a desintegration of the acylated anthocyanins arises that reduces the measured values. A compromise settlement was to use the maximal extinction of a test sample in the area between 550 to 450 nm as measure for the concentration.

For the measurement flowers of the same age were gathered randomly at the same time of day (4 p.m.) and normally the first fully opened flower of an inflorescence of the respective progeny was taken. They were put into a test-tube with water and left overnight at +4 °C in cold storage in order to compensate for possible turgescence differences. Flowers that had been moistened by rain or stained with dirt were dried after cleaning for two hours at room temperature. For the measurement of the fresh weight of each double flower only fully developed, dry, clean and homogeneously coloured petals were used and a quantity of about 80 to 100 mg was weighed out on a balance scale with reading accuracy of ± 5 mg.

For the extraction twenty times the fresh weight of 1% hydrochloric methanol was added, the test-tubes at once closed with rubber stoppers and kept overnight in cold storage at +4 °C.

For the measurement in a recording spectral photometer (Zeiss RPQ II) 2.0 ml extraction liquid were added to 0.1 ml of the extract, well shaken, and measured against the solvent within 550—450 nm. The majority of the extinction values are under 1.0. The measured values used in subsequent publications are multiplied by 10^3.

Zusammenfassung

Es wird ein experimentelles Material entwickelt, auf das das im Teil I dieser Reihe beschriebene mathematische Modell angewandt werden kann. Es besteht aus allen kombinatorisch realisierbaren genotypischen Variationen 4 verschiedener Loci, die in die Modifikation des Anthocyanmoleküls durch Oxydation, Glykosidierung und Acylierung bei der einjährigen Crucifere *Matthiola incana* R. Br. einbezogen sind.

Diese Gene werden vor einem hinsichtlich der Intensität der Blütenfarbstoffe weitgehend isogenen Hintergrund variiert, ihre qualitativen Wirkungen und Wechselwirkungen werden ebenso wie die Zusammensetzung und Isogenisierung des genetischen Hintergrundes beschrieben. Als simuliertes quantitatives Merkmal dient die Gesamtkonzentration der Anthocyane/Petalenfrischgewicht, die im registrierenden Spektralphotometer gemessen wird.

Die genetische Struktur dieses Materials ist Voraussetzung für die Anwendung des früher beschriebenen Modells, die Kenntnis der qualitativen Wirkungen und Wechselwirkungen der experimentell variierten Gene kann die Interpretation der Parameterschätzungen und die Verbesserung des Modells erleichtern.

Acknowledgments

I gratefully appreciate the valuable assistance of Mrs. G. Mezger in translating the manuscript and the correction of the translation of Mrs. Lynn Graf-Felder. Possible errors are nevertheless on my own responsibility.

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Received May 12, 1971

Communicated by H. Stubbe

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