

Genetic Effects on Phenotypic Traits in *Populus* Inferable From an Explorative Analysis of Response Functions (ARF) in a Factorial Mating Design

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Abstract

Full sib families from a factorial mating design of 2 aspen species (*Populus tremula* and *P. tremuloides*) are used to analyse maternal and paternal effects on a number of metrical traits. Against common practice, we explicitly base our analysis on the cause-effect principle manifest in the concept of response functions (norms of reaction). Absence or presence of interaction between the parental effects, modes of operation of the effects (linear, non-linear) as well as hypotheses on their genetic basis are explored with the help of a method of the analysis of response functions (ARF) combining graphical and statistical elements. Besides revealing important and hitherto widely ignored aspects of the variation of parental effects on various traits in their offspring, the results helped to substantiate several criticisms of the analysis of variances (ANOVA) as an analysis of causes. One interesting observation consists in the considerable degree of sexual asymmetry in the parental effects on some of the traits: while paternal effects showed no interaction with maternal effects, strong interaction occurred in the reverse direction. Other characteristics that are rarely, if at all, observable by performance of an ANOVA concern modes of operation of effects, which are readily shown by the present methods of ARF to occur in additive as well as multiplicative form in some of the studied traits.

Key words: *Populus*, analysis of response functions, analysis of variance, factorial mating design, inheritance analysis, modes of gene action, parento effects, sexual asymmetry, interaction.

FDC: 165.3; 165.4; 176.1 *Populus tremula*; 176.1 *Populus tremuloides*.

Introduction

In animal and plant breeding, questions on genetic controlledness of an observed variation and the mode of gene action are usually answered by quantitative methods applied to the distribution of phenotypic trait states. However, two fundamental contradictions occur in the methodology of conventional analysis.

1) The validity of the conventional statistical model used in quantitative genetics depends on the fulfillment of various biological assumptions, which can hardly be tested. The quantitative genetic parameters estimated under the environmental and genetic conditions of a specific experiment and for a specific collection of individuals apply only, if the reproductive system is panmictic. In real biological populations, if at all, this ideal condition might hold true only at a few gene loci.

Our knowledge on the genetic basis of quantitative genetic theory is scanty. The process of the realization of genetic information up to the observable expression of phenotypic traits is widely unknown. It is uncertain how many genes as carriers of this information participate in the genetic control of a trait. On

the one side, a single controlling gene locus is assumed and it is hoped, that the pertinent theory may be extended to several gene loci. On the other side it is known, that the genes contributing to the control of a trait hardly act independently (LEWONTIN, 1977). KEMPTHORNE, a research worker who made profound contributions to quantitative genetics, hints at the weakness of its theory and its basic concepts (KEMPTHORNE, 1988). He proposes the development of new models on the basis of new biological and biochemical findings.

2) The variation contained in the distribution of almost any trait is quantified and its genetic parameters are estimated by the analysis of variances, a method which is well-known and has been widely introduced in biological research. This approach to the analysis of biological variation employs a linear model, which in the case of long-lived organisms such as woody plants, is hardly more justified than a multiplicative or exponential model.

In his account of the analysis of variances and the analysis of causes, LEWONTIN (1974) points out, that analysis of variance is not an appropriate tool for the explanation of functional biological relations. Attempts to quantify phenotypic variation in populations without sufficient knowledge of the underlying reaction norms must remain incomplete, because an analysis of variances confounds genetic and environmental variation (LEWONTIN, 1974).

Moreover, when GREGORIUS (1977) and GREGORIUS and NAMKOONG (1986) developed a new concept for genotype-environment interaction based on response functions, they found that the analysis of variances is no satisfactory method for detecting interactions. A summarizing statement on this subject has been given by SUZUKI et al. (1981). These authors indicate, that in actual fact no partitioning of the variation can partition the causes of this variation, because in producing the phenotype, genotype and environment interact.

If one accounts for these problems involved in the theory of quantitative genetics and analysis of variance, it turns out, that a precise modelling of biological reality is no longer possible, and that a simplified and imprecise modelling forbids itself. However, the employment of norms of reaction (response functions), although rarely applied (GUPTA and LEWONTIN, 1982), recommends itself as a valid alternative for the analysis of genotype-environment interaction. Almost 20 years ago, GREGORIUS (1977) proposed the principle of causation, which has later been extended by GREGORIUS and NAMKOONG (1986, 1987). Yet, even though more recently the idea of response functions with its implied concepts of plasticity has gained some attention, it entered quantitative analysis only to the degree that it is adaptable to the common methods of the analysis of variances (see e.g. DUTILLEUL and POTVIN, 1995, and the literature cited therein).

On the basis of a new reflection on the principle of cause and effect, the present paper proposes a new method in order to approach genetic controlledness and the mode of gene action more closely. The method is demonstrated by results of factorial crosses in aspen.

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Methodology of the Analysis of Response Functions (ARF)

To be able to base the discussion of our experimental results on a direct analysis of hypothetical causes for observable trait variations we resort to the general formulation of the cause-effect principle derived by GREGORIUS and NAMKOONG (1987). In this formulation separability and cooperation of the effects of 2 causal variables X and Y jointly determining the response R of a specified system are considered. Earlier formulations (GREGORIUS, 1977; GREGORIUS and NAMKOONG, 1986) referred to individuals as systems, whose genotypes and environments constitute the 2 causal variables X and Y for their (phenotypic) responses R . The concepts derived from this consideration essentially trace back to the idea of "consistent" effects of causes in the sense that the effect of x on the response variable is consistently distinguishable or indistinguishable (across all Y -states) from the effect of any other X -state. Thus x is said to have a *consistent effect* on R if for any other X -state x' either $R(x, y) \neq R(x', y)$ for all Y -states y or $R(x, y) = R(x', y)$ for all y (in the latter case x and x' have the same effect on the response variable R).

Therefore, the effects of the causal variable X are *separable* from those of the causal variable Y if *all* X -effects are consistent, i.e. if Y -effects do not obscure the differences in response due to X -causes. If such separability is not realized, the X -effects are said to *interact* with the Y -effects. When applied to genotype and environment as causal variables (where the response function of a genotype to its environments is known as the "norm of reaction" [WOLTERECK, 1909]), inseparability of effects is viewed as genotype-environment interaction, and it follows from the above that such interaction may take place only in one direction (unilateral interaction) or in both directions (bilateral interaction). Any causal analysis of response functions proceeding along these lines will be referred to as ARF in the sequel to distinguish it clearly from the common methods of the analysis of variances (ANOVA). In the final discussion a comparison of the results obtainable from both approaches to a causal analysis will be presented.

Causal analysis of response functions specified by distributions of metrical traits

With reference to the present study of full sib families resulting from a factorial mating design, the seed and pollen parents constitute 2 causal variables the effects of which on the performance of the offspring from each cross are to be analysed in a fixed environment. Environment is therefore considered to have a constant effect, which rules it out as a causal variable interacting with genetic effects. In this case the primary response actually consists in the (frequency or probability) distribution of a trait within a full sib family rather than a single measurement taken from a single individual, and the system defining the unit of measurement is composed of all possible offspring from a cross. However, distributional parameters such as expectations, quantiles, variances, diversity, evenness, etc., may as well serve as response functions of full sib families.

It is important to note here, that in the terminology of classical experimental design each particular seed and pollen parent must be referred to as a level of each of two factors. Distributional parameters are then responses to pairs of levels of the 2 factors. The problem arising in this context is one of the precision of measurement as becomes apparent from the necessity to apply statistical methods for estimation of the parameters. A different situation would arise, if each individual offspring were to be considered as the unit of meas-

urement (the system) such that each pair of levels consisting of a seed and a pollen parent would specify definite causes for the individual response only to the extent that the gametic contribution of each parent to its offspring is uniquely specified. For heterozygous parents this is not the case, so that the immediate causes for the response of each offspring may be different and will vary according to probability laws. This creates an ambiguity in the interpretation of the cause-effect relationship and therefore advances consideration of distributions of traits in full sib families as responses to the fixed causes provided by the 2 parents.

Distributional parameters may in fact provide the information relevant to the analysis of the effects of the genetic contributions of parents to their offspring. For example, if a particular seed parent is homozygous at all loci involved in the control of the trait under consideration, then its offspring with any of the pollen parents can be expected to be consistently less variable than the offspring from crosses with a highly heterozygous seed parent. In the same way, if the mode of gene action (i.e. mode of operation of gene effects) were additive, the average performance of offspring from crosses with one seed parent are expected to consistently differ from the offspring from crosses with another seed parent, the difference being of about constant size. Consequently, variances and expectations of distributions of metric traits may reflect distinct aspects of the genetic contributions of parents to their offspring.

Other characteristics of distributions, when considering the nature of the traits they refer to, will reveal further insights into the effects exerted by the parents on their offspring. In the present study we will concentrate on the information obtainable from the average performance of full sib families. Hence, the variability of the gametic contributions of heterozygous parents to a full sib family (and thus the mode of gene transmission) is not reflected in the measurement. On the other hand, irrespective of the number of controlling loci, the mode of gene action may show up in the expectation to the degree to which the linear procedure of averaging does not obscure this mode. Additive gene action belongs to this category in that it produces constant differences in response functions of seed (or pollen) parents. The same is true for multiplicative gene action, where the response functions of two seed (or pollen) parents differ by a constant factor (for random fusion of gametes in crosses).

Classical forms of interaction of genes such as complete dominance may be recognized by a certain invariance in response of the offspring of a particular parent across its mates. In the extreme case of homozygosity for the dominant genes, all full sib families generated by such a parent display the same distribution for the trait under consideration and thus the same average performance. The response function of this parent is therefore constant. For a different parent some of its responses are likely to equal this constant while others don't, so that the effect of either parent on its offspring is not consistent in the above sense. This principle generalizes to any situation where the offspring of 2 seed parents, say, show equal response with respect to one but different response with respect to another pollen parent. The gametic contributions of the first pollen parent may then be considered to suppress manifestation of the difference between the contributions of the two seed parents while the second pollen parent does not suppress manifestation of this difference. Yet, other forms of gene action may be more difficult to recognize directly after averaging over members of a full sib family.

For the present factorial mating design, the conventional methods of the analysis of variances (ANOVA) follow the lines

set by the "experiment II" of COMSTOCK and ROBINSON (1952), where the above-mentioned aspects of average performance are treated in terms of "general combining abilities" of parents and "specific combining abilities" of crosses. In this context, general combining abilities gain relevance in the case of additive separability of the parental effects, while specific combining ability becomes significant for "additive interaction", which in many cases need not imply inseparability of effects. The theoretical foundation of this linearly modeled analysis of biological reality is found in many textbooks (see e.g. FALCONER, 1981; NAMKOONG, 1981; BECKER, 1984) and has also found broad application in forest plant breeding. For the data to be discussed in the present paper GALLO (1985) and GALLO et al. (1985) conducted these conventional analyses.

The canonical representation of the mode of operation of effects

Provided effects of causal variables on response functions are separable and thus definable, it remains to answer the question as to the mode of operation between these effects producing the response. The above mentioned additive mode of operation is the most frequently considered mode, though other modes such as multiplicative and exponential have also received attention (see e.g. POONI and JINKS, 1980; or GREGORIUS and NAMKOONG, 1986).

As was demonstrated for genotype-environment interaction by GREGORIUS (1977), the mode of operation can be made transparent by utilizing the response function of a particular genotype, for example, for representation of the effects of the environmental variable. Later on GREGORIUS and NAMKOONG (1987) showed that the principle of fixing a particular value of one causal variable for representation of the effects of the other causal variable via its response function provides the basis for a canonical representation of both effects and their operation for general cause-effect relations. Even though the implied method of analysis is based on the assumption of separable effects, it can be applied to inseparable effects as well, since the detection of inseparability (inconsistency) is an intrinsic part of the analysis.

The method of analysis is built on response functions specifying for each fixed value of a causal variable the set of responses to the other causal variable, and it consists in

(1) choosing a reference value from one causal variable (a particular parent from the set of seed parents, for example) the response function of which (consisting in the traits of the full sib families generated by the crosses with each of the pollen parents) serves as a scale for the effects of the other causal variable (the pollen parents), and

(2) mapping the other response functions against the reference function.

When applied to a real valued continuous response, this procedure is equivalent to using the reference response as the independent variable in a coordinate system against which the other response functions are plotted on the ordinate. Since in this coordinate system the reference response defines the scale, it re-appears in the line of unit slope passing through the origin. The above-mentioned examples of operations between effects of causal variables actually refer to this representation, where additivity shows as a set of parallel straight lines of unit slope, and where multiplicativity results in a bundle of rays with a common point of intersection (GREGORIUS and NAMKOONG, 1986).

Once the reference response function defining the scale of the coordinate system is specified, the effects of the causes (belonging to the same causal variable) determining the other response functions are separable (consistent) if the response

functions do not intersect, and the effects of the other causal variable are separable if the functions are strictly monotone (GREGORIUS and NAMKOONG, 1987). Deviations from these characteristics indicate unilateral or bilateral interaction of effects of the causal variables. This canonical method and its implied graphical representation has been previously applied to the analysis of genotype-environment interactions in tissue cultures (GLOCK and GREGORIUS, 1986; GLOCK, 1989). The method will also provide the explorative basis of the present analysis of data.

Statistical aspects of separability

The statistics of separability of effects is not yet developed, which explains why we refer to the following analysis of our data as "explorative". Yet, some provisional statistical tests of separability of effects on response averages can be performed. With frequency distributions as responses, the problem basically consists in providing methods of testing differences between or identity of such distributions. The latter clearly is statistically infeasible, so that non-significance in a statistical test can only provisionally be accepted as indicating indistinguishability of effects. In the particular case of a continuously varying metrical trait, such as the averages (expectations) to be considered in the following, an additional opportunity for the detection of interaction is provided by statistically significant changes in ranking between 2 response functions.

In this situation, sign tests could provide an appropriate testing procedure (GREGORIUS and NAMKOONG, 1986). Alternatively, it suffices to observe that the difference in averages between 2 levels of 1 factor are positive for 1 level of the second factor and that this difference is negative for another level of the second factor, in order to classify the effects of the first factor as interacting with the effects of the second factor given both differences are statistically significant with respect to STUDENT's *t*-test, for example. This testing procedure has been applied previously by GLOCK and GREGORIUS (1986) to observations from tissue culture experiments and will also be applied in the following analysis, where seed and pollen parents serve as factors and average performance of a full sib family in a specified environment defines the response variable.

Application to a 7 x 7 Factorial Mating Design of Aspen

Material

Our experimental object consists of European (*Populus tremula*) and North American (*P. tremuloides*) aspen. Eight parental trees of European and 6 of North American aspen as well as the offspring produced according to a 7 x 7 factorial mating design entered the investigations. Detailed information on origin and designation of the related parental trees is found in GALLO (1985) and GALLO et al. (1985). The majority of the parental trees was selected in an unsystematical way for growth or form in their original sites. Three of the 14 clones are offspring from open pollination of an unselected seed parent.

Methods

The 49 full sib families resulted from controlled crosses according to the mating scheme specified in table 1. The crosses were performed on twigs maintained under hydroculture in a heated green house (WETTSTEIN, 1933).

The offspring were measured at different ontogenetic stages (seed, seedling, plant) in the respective germination, nursery, and field trials (up to the age of 7 years). A number of 23 different metric traits were determined, which were grouped

Table 1. – Mating design and numbering of full sib families.

		δ						
		<i>P. tremula</i> (a)			<i>P. tremuloides</i> (b)			
		W 52	W 66	CVS 52	Ihl 1	T 44-60	T 428	Tur 141
\varnothing	(a) Brauna 11	1	2	3	4	5	6	7
	(a) Gr.Dubr. 1	8	9	10	11	12	13	14
	(a) W 51	15	16	17	18	19	20	21*
	(a) W 95	22	23	24	25	26	27	28*
	(a) C 61	29	30	31	32	33	34	35
	(b) Ihl 3	36	37	38	39	40	41	42*
	(b) Ihl 5	43	44	45	46	47	48	49

*) = crossings in 1982

Table 2. – Summary of the studied metrical traits.

trait type	trait	time of observation
juvenile traits	1 thousand seed weight	after collection
	2 germinability	
	3 germination speed	
	4 pre-germination abnormalities	
	5 post-germination abnormalities	
	6 abnormality due to absence of radicle	
morphological traits	7 hairiness	1 st year
	8 color of terminal leaf	2 nd year
	9 bicolored shoots	2 nd year
	10 branch angle	6 th year
	11 stem straightness	7 th year
phenological traits	12 flushing	2 nd , 6 th and 7 th year
	13 growth termination	1 st and 6 th year
	14 leaf fall due to growth termination	1 st year
growth traits	15 height	3 rd month plus 1 st , 2 nd , 3 rd , 5 th , 6 th , 7 th year
	16 breast height diameter	6 th and 7 th year
	17 number of branches	2 nd and 7 th year
	18 crown diameter	7 th year
stress traits	19 <i>Melampsora</i> attack	1 st and 2 nd year
	20 leaf fall due to shading	2 nd year
	21 <i>Saperda</i> attack	6 th year
	22 growth termination due to drought	7 th year
	23 family viability	7 th year

into 5 types of traits according to functional criteria and which are summarized in table 2. Descriptions of the details of measurement of the traits can be taken from GALLO (1991).

Among the 23 traits listed in table 2 only 6 will be used for demonstration of the power of the ARF: germinability, hairiness, flushing, height at year 7, *Melampsora* attack, leaf cast due to shading. Furthermore only those results will be presented which have relevance in the application of the ARF. A detailed presentation of the experimental results can again be taken from GALLO (1991).

Statistical analysis

As was mentioned above, the response functions to be considered in the present study are defined by the expectations of various metric traits measured in full sib families, and the expectations are estimated by the arithmetic means in samples taken from the respective families. If admissible, the means for field traits were taken over 2 field experiments (GALLO, 1991).

Consideration of distributional parameters other than the expectation, such as the median, might have been more useful in the present as well as in other works since they are less sensitive towards asymmetric distributions, for example. However, for practical reasons concerning availability of only small sample sizes from some families, analysis of some traits from plot observations, or homogeneity and comparability of the results obtained within the present study as well as with those of other studies, we decided in favour of the expectation as estimated by the sample mean. Moreover, as was argued above, the linearity of the statistical expectation operation guarantees

that characteristics of the mode of operation of effects (such as the mode of gene action) are reflected more directly in the observations. The structure of the present experiment together with the applied analytical method is then suitable for the detection of genetic effects on trait variation provided maternal or paternal effects are separable.

For all statistical analyses the level of significance was fixed at 0.05. Despite the fact that the ARF is conceptually based on the validity of the cause-effect principle, we do not discuss here the scientific justification of "randomness" in statistical inference but rather assume that it can be interpreted in a way (e.g. in terms of chaotic transitions) which is compatible with deterministic views. The statistical analysis of the data and their graphical representations was conducted with the help of the SAS-SYSTEM (1985 a, b and c).

Results

Germinability

In general, both the response functions of the seed (\varnothing) parents over all pollen (δ) parents and those of the pollen parents over all seed parents display strong interactions between their effects (Figure 1a and 1b).

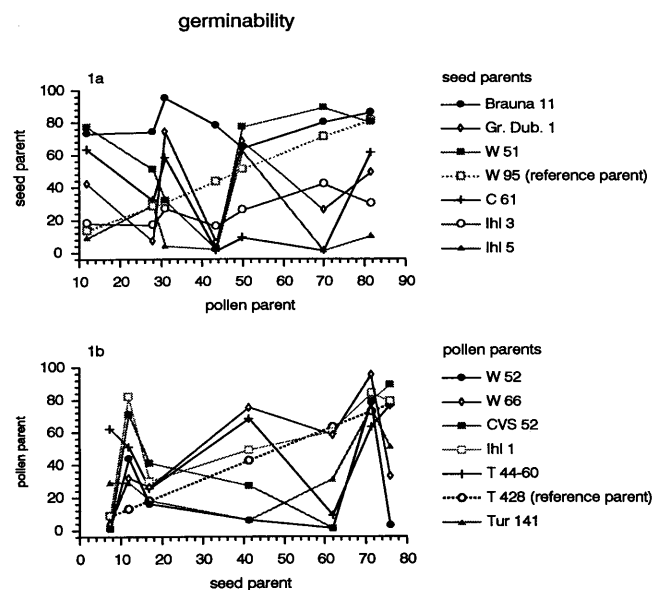


Figure 1. – Separability of the genetic effects of \varnothing (a) and δ (b) parents on seed germinability 3 months after collection.

However, the response functions of the seed parents Brauna 11, W 95 and Ihl 3 differ considerably (Figure 1a); this difference is also statistically significant. Considering these 3 half-sib families, the values of Brauna 11 are consistently large, those of W 95 consistently average, and those of Ihl 3 consistently small. Hence, the effects of these three seed parents are separable from those of the pollen parents. Also, \varnothing Ihl 5 could be assigned to this group unless its interaction with δ T 44-60 had produced such a large value (Figure 1a). The half-sib progeny of \varnothing Brauna 11 differs significantly in its response function from \varnothing C 61 and Ihl 5. \varnothing Ihl 3 varies statistically significant from the response function of \varnothing W 51, the latter also from \varnothing Ihl 5.

Among the pollen parents, only 2 pairs of response functions differ significantly, i.e. δ W 52 from δ Ihl 1 and δ Ihl 1 from δ Tur 141 (Figure 1b).

Hairiness

The remarkable consistency of the response functions of the seed parents over all pollen parents is statistically significant (Figure 2a). The low number of only 3 classes of scores was sufficient for demonstrating the differences between the seed parents. The progeny of ♀Ihl 5 and ♀Ihl 3 had hairless third internodes of their stems. The progeny of ♀C 61 were most hairy. All response functions except the following were mutually significantly different: ♀Ihl 5 and ♀Ihl 3, ♀W 51 and ♀Brauna 11, ♀W 51 and ♀W 95, and ♀W 51 and ♀Ihl 3.

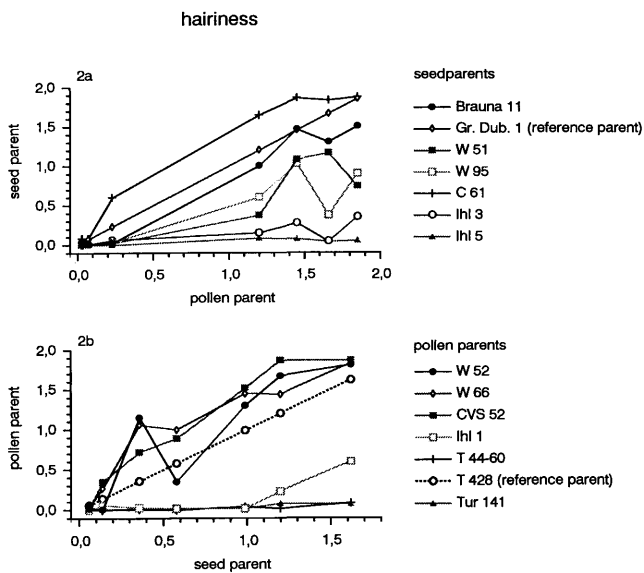


Figure 2. – Separability of the genetic effects of ♀ (a) and ♂ (b) parents on hairiness of the third stem internode of the first-year seedlings.

Even for these exceptions there exist subsets of pollen parents restricted to which the maternal are separable from the paternal effects. Hence, effects of female parents are clearly separable from those of the male parents (Figure 2a). Among the seed parents, the curves indicated the multiplicative form of separability (GREGORIUS and NAMKOONG, 1986) because of the increasing divergence of the curves with increasing effects of the pollen parents.

The 2 groups among the response functions of the pollen parents (Figure 2b) were statistically significant. The progeny of ♂Ihl 1, ♂Tur 141, and ♂T 44-60 had almost no hairs, while those of the other pollen parents had many. The individual functions within the first group did not differ significantly. Within the second group, the progeny of ♂T 428 differed significantly from those of ♂W 66 and ♂CVS 52. The response functions of ♂T 44-60, ♂T 141, and partially also those of ♂Ihl 1 were consistent over almost all ♀ partners and thus separated multiplicatively from the others. The other functions were more or less linear if compared with the reference pollen parent, T 428.

Flushing date

Flushing was observed using on one occasion a scoring system with 6 steps ranging from 1 (closed bud without elongation) to 6 (unfolded leaves). Due to this procedure, late flushing of the seedlings leads to a low average.

The effects of both female and male parents remained consistent over the years. The analysis of the graphs revealed no interaction between the response functions of both sexes or between parents and years. Moreover, the response functions of

female and male parents were consistent. The ranks of the half-sib families remained the same in the 2 experimental locations, and the ranks of the full-sib families remained largely the same. Consequently, there was no interaction between genotype and environment. Also, the response functions of the 2 experimental locations were consistent over all female and male parents. These response functions did not differ for flushing date at the age of 6 years, but they did so one year later, when the trees flushed earlier in experiment As93 than in experiment As95.

Because of the above-mentioned consistencies, it is possible to judge the behavior of this trait from the results in one representative year. Genetic analysis is therefore restricted to the results at the age of 6 years.

The response functions of the seed parents were consistent, as has already been mentioned (Figure 3a). The ♀Ihl 3 and ♀Ihl 5 with a positive effect on flushing date differed significantly from the other seed parents except ♀W 51. The latter parent produced early-flushing progeny after pollination by *P. tremuloides*, whereas it retarded flushing of the progeny after pollination by *P. tremula*. The late-flushing ♀Gr.Dubr. 1 had a consistent retarding effect over all pollen parents.

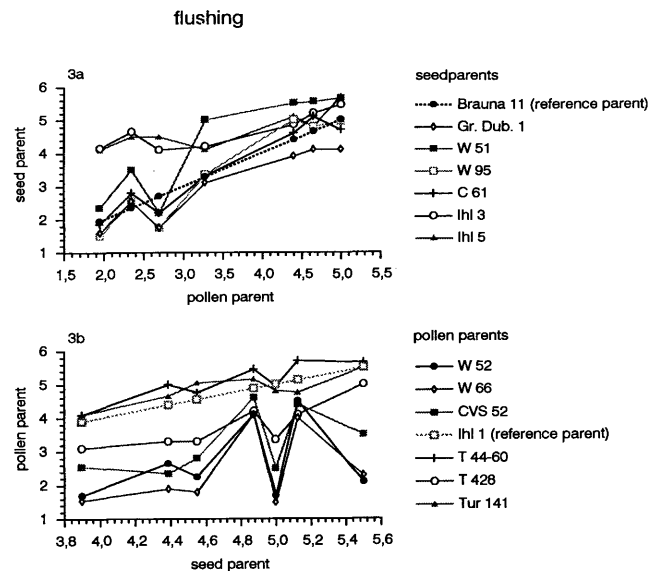


Figure 3. – Separability of the genetic effects of ♀ (a) and ♂ (b) parents on flushing date at the age of 6 years.

As to the consistency of the response functions and the statistical significance of interactions, the response curves of the seed parents were relatively separable from those of the pollen parents (Figure 3a). Although the shape of the response curves is mostly linear, the functions of the significantly different groups diverge multiplicatively. The separability of the response functions was lower among the seed parents than among the pollen parents (Figure 3a and b). In figure 3b, 2 statistically different groups are clearly visible, i.e. ♂Ihl 1, ♂T 44-60, and ♂T 141 produce progeny with early or average flushing date and the others sire late-flushing progeny. The curves in the first group were not significantly different, whereas within the second group, ♂T 428 and ♂CVS 52 differ significantly from ♂W 52 and ♂W 66. In the sense of statistics, 3 significantly different groups exist. The interaction between female and male effects was weak. ♀W 95 induced late flushing after pollination by *P. tremula* or ♂T 428. The response curves were more or less linear.

Tree height at an age of 7 years

In this trait, the effects of the female parents could not be separated from those of the males (Figure 4a). Only the partial consideration of ♀C 61 and ♀Brauna 11 leads to separability of these 2 seed parents from their male crossing partners. If the response functions of all seed parents are considered, many interactions can be detected graphically. Some of these are statistically significant. The response function of ♀C 61 differed significantly from those of ♀Brauna 11, Gr.Dubr. 1 and ♀W 95. Other statistically significant differences could be detected between ♀W 51 and ♀Brauna 11, and between ♀W 51 and ♀Gr.Dubr. 1. During earlier height measurements, ♀Ihl 5 had displayed the very large response values. It retained its superior behavior after pollination by male *P. tremula* and by ♂T 428. However, it gave rise to less tall progeny in combination with ♂T 44-60, ♂Tur 141, and ♂Ihl 1.

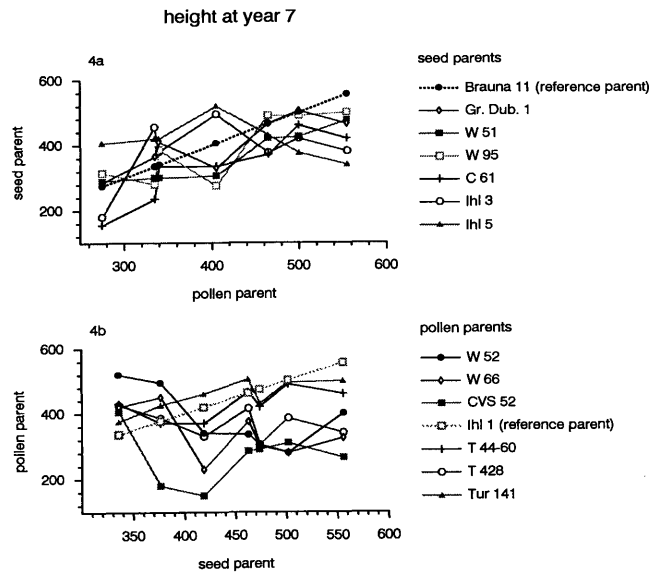


Figure 4. – Separability of the genetic effects of ♀ (a) and ♂ (b) parents on height growth at the age of 7 years.

The response functions of the pollen parents displayed larger consistency than those of the seed parents (Figure 4). However, only ♂CVS 52 with lower values was consistent over almost all seed parents and differed significantly from the other pollen parents except ♂W 66 and ♂T 428.

If one considers only female *P. tremula*, 2 clearly separate groups can be detected. ♂Tur 141, ♂T 44-60, and ♂Ihl 1 induce larger response values, the others sire less tall progeny. After crossing ♀Ihl 3 and ♀Ihl 5, the 2 female *P. tremula*, ♂W 52 and ♂W 66 induced fastest height growth. This means, that the fastest-growing full-sib families except those sired by ♂CVS 52 and ♂T 428 are interspecific hybrids. Accordingly, the largest response values were either due to *P. tremula* or *P. tremuloides* parents. Some significant interactions between female and male effects were also found.

Melampsora attack

The response functions of ♀Ihl 3 and ♀Ihl 5 were consistently separable from and significantly different from the others over all pollen parents (Figure 5a). These 2 showed lower response values (indicating less severe attack) and did not differ significantly among each other. If one considers 2 groups of seed parents, i.e. these very 2 females vs. the others, their maternal effects are clearly separable from the paternal effects. The consistency of the response functions of the male parents

can already be suspected from the monotony of the response functions of the female parents. Also, ♀Gr.Dubr. 1 differed significantly from ♀W 51 and the reference parent, ♀C 61.

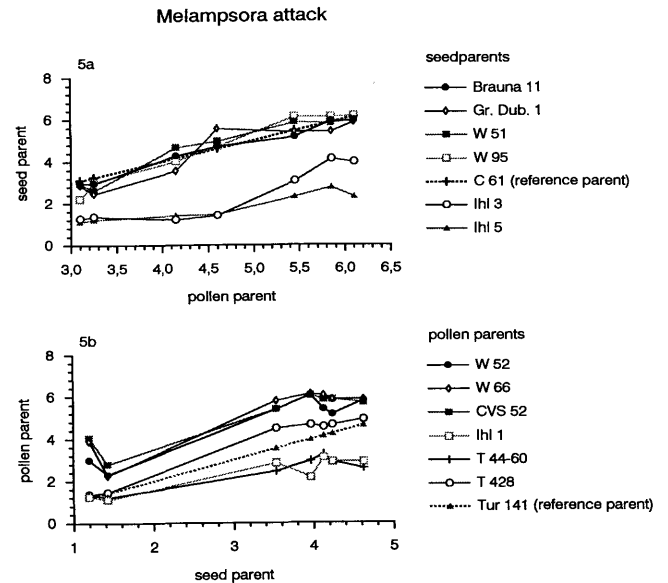


Figure 5. – Separability of the genetic effects of ♀ (a) and ♂ (b) parents on resistance to *Melampsora magnusiana* during the second vegetation period.

The response functions of the pollen parents display more discriminant effects than those of the seed parents (Figure 5b) and approached linearity more closely. Only some of all possible contrasts are not significant. These are ♂CVS 52 compared with ♂W 52 and ♂W 66, and ♂Ihl 1 compared with ♂T 44-60. Four consistent functions can be detected: (a) the susceptible group comprising ♂W 52, ♂W 66, and ♂CVS 52, (b) ♂T 428, (c) ♂Tur 141, and (d) the very resistant ♂Ihl 1 and ♂T 44-60. According to these groups, the effects of the male parents are clearly separable from the effects of the female parents, at least more clearly than vice versa.

It can also be seen from figure 5, that half-sib families having *P. tremuloides* either as their female or male parent are less infected, and that the half-sib families sired by various *P. tremuloides* males differ significantly. ♂Ihl 1 and ♂T 44-60 produced more resistant progeny than ♂Tur 141, and these 3 sired more resistant progeny than ♂T 428. Significant interactions can be detected neither in figure 5a or b.

Leaf cast due to shading

The response functions of the seed parents indicate clear separability of the female from the male effects (Figure 6a). These functions approach an exponential form. No strong interactions were found. Two significantly different groups exist: ♀Ihl 3 and ♀Ihl 5 produced progeny with big losses of leaves after pollination by any pollen parents and thus differed from the rest. All progeny of ♀Ihl 3 except those after pollination by ♂CVS 52 shed more leaves than those of ♀Ihl 5.

Among the response functions of the pollen parents (Figure 6b), the consistency of the effects of ♀T 44-60 becomes apparent. Greater part of the lower leaves of its progeny had been shed at the time of observation. This consistence turned out to be statistically significant. The effects of the other pollen parents were mutually not significantly different, although the effects of ♂Ihl 1 and ♂Tur 141 in combination with the effects of some female parents clearly differ graphically. The

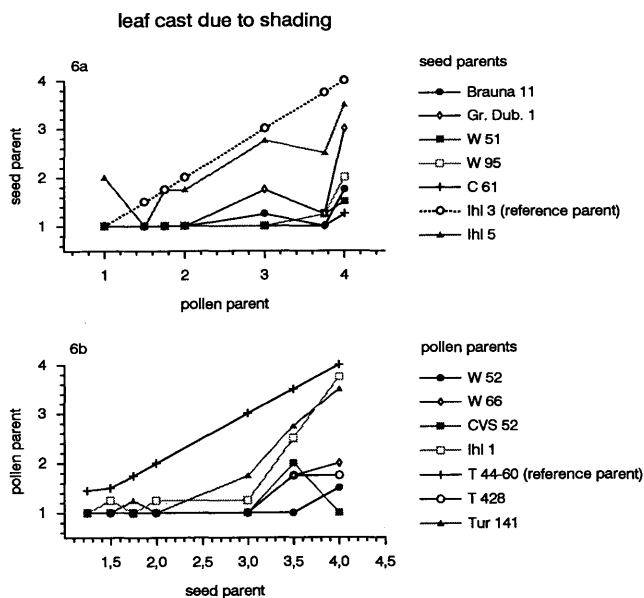


Figure 6. – Separability of the genetic effects of ♀ (a) and ♂ (b) parents on leaf cast at the age of 2 years.

significance of some moderate interactions could unfortunately not be tested statistically because of the scoring procedure. The response curves of the pollen parents approached the exponential form more closely than those of the seed parents.

Discussion

Inheritance analysis of metric traits

As has been indicated above, the estimation of quantitative genetic parameters from progeny tests faces several conceptual problems. Nevertheless, almost all of our experience in breeding derives its understanding from this inferential basis. Since the present graphical method of explorative validation of genetic control and mode of gene action proceeds from a different paradigm and seems to be unique at least within forest plant breeding, some additional clarification might be in order. To prevent possible misunderstanding it should, however, be emphasized that our critique of the traditional paradigm of quantitative genetics concerns the basic hypothesis underlying its models. Modelling itself is thus not a matter of disagreement on our side, it rather is the indifference to the fact that we are modelling the unknown.

More specifically, if an experimental design allows us to estimate the average (marginal) effect of a specified gene or set of genes across a specified set of genomes (genetic backgrounds) and environments, we tend to model this effect as a constant and possibly small contribution recognizable over this set of genomes and environments (NAMKOONG et al., 1988). It is probably this idea that led to the common assumption that continuously varying metric traits be “controlled” by several genes each with small effect. This assumption, however, is known not to be applicable to a number of metrical traits in animals (FAMULA, 1986) and plants (FIRKMANN, 1974). Individual genes may well exert major effects on growth traits in animals (FAMULA, 1986). Even the variation of traits such as age at death, which is commonly thought to be under polygenic control, has been shown in *Drosophila melanogaster* to be in accordance with a 2-locus mode of inheritance (YONEMURA, 1989). Similarly, PRUDIC (1981) advances the hypothesis of a 2-locus control of the variation observed for height at year 1 in aspen and hybrid aspen.

The observations presented on the variation of metric traits in the present paper could not consistently be explained by simple MENDELIAN modes of inheritance, which supports the assumption of control by a larger number of loci. However, particularly for scored traits, where variation is more or less arbitrarily broken into discrete classes and where the scale of scoring is limited, an actual control by a small number of loci could possibly not have been detected. In addition, it has to be recalled that in the present analysis the participation of the total genome (including the “genetic background”) in the control of both qualitative and quantitative traits is part of the conceptual approach.

Modes of gene action analysed by response functions

At a first sight, the present approach of combined graphical and statistical ARF may appear to be inferior in methodological rigour to the well established methods of estimating and testing quantitative genetic parameters on the basis of the ANOVA. In fact, the ANOVA seems to have almost reached the state of self-evidence in the causal analysis of metrical traits. Yet, the results of our analysis clearly demonstrate the capacity of our approach to uncover aspects of genetic control and modes of gene action of a trait that are missed by application of methods of the ANOVA.

For example, the maternal and paternal response functions showed significantly non-linear behaviour for some traits but remained separable. Interaction between paternal and maternal effects is thus absent, which cannot be verified by ANOVA methods because of the non-linearity of the response. This emphasizes a fundamental difference between the 2 approaches consisting in the fact that in the ANOVA the (linear) mode of gene action is stipulated while in the ARF it is analyzed. Biological reality may thus be reflected more closely by the ARF at least to the degree that it avoids misconceptions resulting merely from the arbitrariness of scaling. The generally observed high discriminative power of the ARF as compared to the ANOVA is likely to be a consequence of this property.

A comparative summary of the results obtained from the ARF and the ANOVA is given in table 3 for some selected traits. The ANOVA data for germinability and *Melampsora*

Table 3. – Comparison of results on the breeding design from ARF and ANOVA for some selected traits.

trait	cause	ARF separability interaction	ANOVA (MQ)
germinability	seed parents	medium	1.864*
	pollen parents	low	0.668 ns
	♀ × ♂	high	0.314*
	♂ × ♀	high	
<i>Melampsora</i> attack	seed parents	high	42.52*
	pollen parents	higher	40.56*
	♀ × ♂	no	0.79*
	♂ × ♀	no	
hairiness	seed parents	higher	4.72*
	pollen parents	high	6.10*
	♀ × ♂	no	0.50*
	♂ × ♀	no	
height at 7 years	seed parents	low	42053*
	pollen parents	medium	193725*
	♀ × ♂	medium	50092*
	♂ × ♀	low	

MQ = mean square, *) = significant ($\alpha = 0.05$), ns = not significant

attack are taken from GALLO (1985) and from GALLO et al. (1985), respectively. For the trait "height at year 7" the results from the ANOVA are included only for 1 field experiment (AS95). The results obtained for the other field experiment (AS93) follow about the same pattern.

The distribution of the original data on hairiness did not allow for a transformation into normality by any of the standard methods. An ANOVA was therefore conducted for the untransformed data, which, in a strict sense, prohibits interpretation of small significant differences. The considerable sizes of the F -values, however, may indicate sufficient robustness against the condition of normality. This is not meant to conceal the fact that, due to its intrinsically restrictive conditions, the ANOVA might be applicable to considerably fewer studies than is actually done in the biological technical literature.

The results on germinability clearly demonstrate that despite considerable bilateral interaction between maternal and paternal effects, maternal effects have a discernible share in the variation of this trait. The mode of gene action can therefore be expected to be largely characterized by inseparability, while the genetic effects of some seed parents appear to be quite consistent. The average of the half sib family of clone Brauna 11, for example, is about 77% and thus exceeds the grand population mean by about 36%. The comparatively high consistence in the response of this seed parent (Fig. 1) thus suggests it for breeding programs aiming at a significant increase of the population mean.

Our observations on hairiness suggest a strong genetic component which is characterized by pronounced multiplicative operation of genetic effects. The response functions of the seed parents and to some degree the pollen parents form a multiplicative pattern with consistent effects for all parents. A provisionally conducted ANOVA produced highly significant interaction among seed and pollen parents despite negligible interaction in the graphical representation. Again, neither the linearly modelled ANOVA nor the associated estimation of quantitative genetic parameters produced results of the specificity obtainable from the ARF.

Flushing appears as another trait under strong genetic control with distinctly separated response functions approaching an additive mode of operation of the genetic effects. The strong genetic control was confirmed by consistent maternal and paternal effects of ramets of different age during 3 years of observation at 2 sites.

The comparatively high separability of paternal effects on tree height persisted over years. In contrast, significant interaction was found for the effects of the seed trees indicating sexually asymmetric contributions to the genetic control of this trait. This situation of unilateral separability of effects provides another example of causal variables detectable by an ARF but not by classical ANOVA. Selection among the pollen parents is thus likely to yield larger breeding success than selection among seed parents, however, the design of production populations has then to account for the specific combining abilities of both parents. In fact, we found both separable and non-separable effects for all of the studied growth traits, where non-separable genetic effects appeared to have a greater share in breast height diameter than in height.

Resistance towards leaf rust caused by *Melampsora* (presumably *M. magnusiana*) also appears to be under strong genetic control. This holds for both aspen species *Populus tremuloides* and *P. tremula* with the tendency for the latter to be more sensitive and for the former to show more pronounced separability of the male effects. Even though the ranking of

response functions was inconsistent for some seed and pollen parents, no significant interaction could have been established. These results suggest that under the environmental and genetic conditions realized in the trial, resistance behaves like a quantitative trait controlled by genes with (probably additively) separable effects. Yet, it should be taken into consideration that continuous variation need not be the result of polygenic control. For example, in a diploid organism, a trait which is additively controlled by the genes at three polymorphic loci realizes at least 27 different states. The distribution of these traits to the usually considerably lower number of scores for resistance or sensitivity may thus closely resemble a continuously varying trait.

As the result of an ANOVA the same data turned out to be in accordance with a strongly expressed additive mode of gene action (GALLO et al., 1985). The interaction component of variance was quite small but significant. The maternal and paternal components of variance explained about the same fraction of the total variance where the maternal component slightly exceeded the paternal component. This contrasts with the statistically supported graphical result of the ARF stating larger separability for the paternal effects. This important information for breeding cannot be obtained from an ANOVA, which points at its limited discriminative power (GLOCK and GREGORIUS, 1986).

Among the stress traits, leaf cast due to shading together with *Melampsora* attack turned out to be highly discriminative. The response functions for these traits showed frequently consistent and separable effects of the parents. Hence, (marginally operating) genetic effects are likely to participate in shaping the pattern of variation, and the mode of operation of these effects is more of an exponential rather than of an additive type. Even if the data would have been transformed in order to meet more closely the requirements of an ANOVA, the linearity of the model would still have made it difficult to extract the biological reality showing in the exponential operation of effects.

Traits with strong bilateral interaction in the ARF showed analogous tendencies in both the ANOVA and ARF. This follows from a comparison of the present results for germinability and thousand seed weight with those obtained for the same data set from an ANOVA by GALLO (1985). Both methods state strong maternal effects on germinability and considerable paternal effects on thousand seed weight.

On the other hand, if the ARF yielded distinct separability of maternal and paternal effects, the ANOVA occasionally produced significant interaction. This is expected in cases of non-additive operation of effects such as observed for hairiness (with a presumably multiplicative operation of effects). Yet, for the largely additive effects on *Melampsora* attack (GALLO et al., 1985) this is disturbing. Thus, depending on the applied method, the genetic interpretation and the decision on breeding strategies would end up with different results.

Moreover, the concept of unilateral interaction introduced by GREGORIUS (1977) enables detailed analysis of effects so far not treated by the methods of the ANOVA. An example is provided by height and other growth traits where interaction was more pronounced in maternal than in paternal effects. The superior discriminative capacity of the ARF also extends to the interpretation of the size of maternal or paternal effects (as became apparent for *Melampsora* attack, hairiness, or recognition of genotypes showing early flushing) as well as to the possibility of partial analyses of response functions.

A carefully conducted ARF may help in overcoming critical assumptions, such as sexual symmetry, made in the models of

quantitative genetics in order to allow the estimation of genetic parameters (FALCONER, 1981). As was shown by GALLO (1991), this assumption is not realized in studies of enzyme gene loci performed in the same material. The studies also revealed strong deviations from HARDY-WEINBERG proportions as well as gametic phase disequilibria.

These as well as other assumptions, which can rarely be verified in experimental designs, shed doubt on the significance of many of the estimates on additive or non-additive components of variance, genetic correlations, selection gains, etc., communicated in the technical literature. It appears that, in the last analysis, such estimates frequently give the impression of precise quantifiability where, more appropriately, they should be taken as indicators of tendencies. At least in such situations, an ARF as presented in this paper might be preferable.

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Natural Poplar Resources in China and Their Significance for Breeding and Afforestation

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Summary

China has a distinctly varied flora, especially in the subtropical mountain regions in the South. This is particularly the case with the indigenous poplars, for example in the Southern and Western regions of the Qinghai-Tibet plateau.

The natural forests were however displaced to a very great extent by various anthropogenic influences in earlier times. Lack of forests and shortage of wood have led in many parts of the country to the establishment of monoclonal plantations with fast growing tree species. But these have created unstable forest structures with a high cultivation risk.

The aim of the Chinese-German cooperative project begun in 1984 is both to preserve still existent natural poplar sources and also to use them for breeding and afforestation purposes. Attention is paid to achieving a well balanced relationship of timber yield to yield stability. Great importance is attached to building up multiclinal varieties with high growing potential and good adaptation to the continental, semi-arid climatic conditions in the project region.

In the course of the project up till now numerous fast-growing and healthy sources of 19 indigenous poplar species and 3 natural species hybrids have been recorded in nine provinces and autonomous regions of Central and North China. Preservation measures have been started. In the project centre Jinshatan extensive living collections have been assembled

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