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ENVIRONMENTAL EFFECTS ON GROWTH OF TISSUE CULTURES OF A WOODY SOLANUM SPECIES (*SOLANUM LACINIATUM*)

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Tissue culture experiments were conducted with four genotypes of *Solanum laciniatum* from New Zealand. Environmental effects on the character "callus growth" were studied for two factors (medium type and a temperature-light combination) at each of the four medium types. These studies were applied to all the four genotypes, and their response functions were analysed. On Sommer media the genotype effect interacts completely with the environmental effects. On Murashige and Skoog media the effect of the non-related genotype was consistently distinguishable from the effects of those genotypes descending from self-pollination, i.e. on MS media the genotypic effect can be observed but it is masked on SO medium. This separability of effects is not influenced by the other environmental situations such as hormone concentrations, light or temperature.

Key words: genotype-environment-interaction; *Solanum;* tissue culture; separability of effects

Introduction

Each character expression is the result of the cooperation between genotype and environment. The problem, however, arises as to determine the degree to which both causes influence the trait expression and whether the effects of the causes are definable.

The experiment demonstrated here shows the interaction or separability of both effects during the expression of the character callus growth. The model of Gregorius and Namkoong [1] on cause-effect-analysis was applied. This concept can be used for the interpretation of all kinds of cause-effect mechanisms because it allows interpretation of the qualities of the effects in terms of separability (i.e. the effects are either consistently different or consistently identical over the range of observations) or of interaction (i.e. the effects are not consistent and therefore not distinguishable over the range of observations).

Materials and methods

The material

The source material of the initial explant was 1-year-old seedlings of a perennial solanum species (*Solanum laciniatum*) from New Zealand. Three genotypes G2, G3 and G5, are full-sibs from self-pollination, whereas genotype G1 has no known genealogical relationship to them. Callus cultures from cambium of internodes were established. Each experiment was repeated with 20 replicates.

The concept

For the interpretation of the results in terms of a cause-effect principle, see Gregorius and Namkoong [1]. A version adapted to the present purpose (genotype-environment-interaction) is given Glock and Gregorius [2]. The results are presented as (stochastic) response functions of the genotypes (= G-response or reaction norm) and as response functions of the environments (= E-response).

For definition and explanations of the terms "separability" and "interaction" in detail as qualities of the cause-effect-system see Gregorius and Namkoong [1] and Glock and Gregorius [2]. The basis of the concept is characterized by the term "distinguishability of genotypic and environmental effects". These are described with the help of "response func-

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tion". which specify the reaction of a particular genotype or environmental situation as causes for the phenotype.

Thus, if one or a group of genotypes differ completely in their response functions ("norms of reaction") to the environmental conditions tested, then their (genotypic) effects are said to be consistently distinguishable or the effect is called to be consistent. If all genotypes have consistent effects then we call it separability of genotypic effects. Analogously, the same is true considering environmental effects when applying environmental response functions.

Interaction means that the effects are not consistent and therefore not separable. For example, if two response functions differ in one case and do not so in another, then the effects are said to interact with one another. Thus separability and interaction are complementary notions.

This way of interpretation allows a new view on a well known problem: this concept enables the discrimination and independent interpretation of the genotypic and the environmental effects. It provides an opportunity to weigh the influence of both the causes on the particular variable trait.

The graphs

The performance of the response functions depends on the choice of any reference. Of course, it must be one of the group of the functions under consideration. The measurements of this reference are arranged with increasing values. Therefore it is always a straight line and fixes the environmental situations or the genotypes. Relative to this reference, all other response functions are drawn, so that the comparable measurements of each genotype in a certain environment are arranged in a vertical manner. Concerning the G-responses, the sequence of environments on the abscissa is given by the chosen reference and will change with changing reference out of the set of the response functions under consideration. But the consistency or inconsistency of the response functions is untouched by such a change because the real value of the certain functions are uninfluenced, naturally. This is true for both types of graphs, the G-response and the E-response.

Considering a graph of G-response functions, the points on the abscissa represent the environmental situations (env. 1 to 16) or selected

Table I. Environmental conditions.

Hormone conc. IAA/BAP	Murashige-Skoog type (MS)		Sommer type (SO)	
	M 1 (0.1/0.5)	M 2 (2.0/0.2)	M 3 (0.1/0.5)	M 4 (1.0/5.0)
10 °C continuous light	Env. 1	Env. 2	Env. 3	Env. 4
20 °C continuous light	Env. 5	Env. 6	Env. 7	Env. 8
20 °C long day 16 h:8 h	Env. 9	Env. 10	Env. 11	Env. 12
30 °C continuous light	Env. 13	Env. 14	Env. 15	Env. 16

parts of them. The numbers on the ordinate reflect the four genotypes (G1, G2, G3, G5). The reverted form is true for the E-response: the abscissa represents the genotypes and the environmental situations are plotted on the ordinate. The environments are grouped as shown in Table I (see next section).

The environments

The medium types were two modified Murashige-Skoog [3] media (M1, M2) and two modified Sommer [4] media (M3, M4). In addition, both medium types contained 500 mg/l cellobiose, 1000 mg/l caseinhydrolysate, and 30 g/l sucrose. For hormones and their concentrations see Table I. Three temperatures (10 °C, 20 °C and 30 °C) were tested for all media and light conditions. The temperature 30 °C was chosen to test whether *Solanum laciniatum*, a subtropical species, needs a higher temperature than autochtonous plants. Continuous light (1000 lux) was used for all temperatures; in addition there was a condition of long-day (16 h of light) with 20 °C. Calli of all four genotypes were grown in each environmental combination as specified in Table I.

Thus, the environments considered consisted of two factors, the media (M1, M2, M3, M4) and the temperature-light combination. The different composition of the hormones was part of the experimental design; M1 and M3 represent the different medium types, whereas the different hormone action could be tested on M2 (the standard medium for maintaining callus) and M4 (a medium thought to induce microshoots).

The character

The observed character was "callus growth". At the beginning of the experiment the initial volume was determined in millilitres displaced water. The same was repeated at the end of 4 weeks. Each growth measurement represents the calculated multiple related to the applied volume at the start of the experiment. The test used in Figs. 1—3 to guarantee statistical independence or identity was the *t*-test.

Results and discussion

The response functions of four genotypes in different environments (up to 16) and the influence of the medium type (MS and Sommer) on the character callus growth was investigated. In Fig. 1a the response functions of all four genotypes show no separability in all 16 environments, except in a small part (see the right half of the graph), where the response function of G1 is separated from the other. However, for the E-response (Fig. 1b) interaction takes place for all the response functions of the different environmental conditions.

Results are similar for growth on Sommer media where separability is absent. Although G2 ranges almost above the others in all environmental conditions, interaction of both effects is present, i.e. there is no separable effect neither in the G-response (consistency is hurt in the environments 4 and 16, even concerning G2) nor in the E-response, where interaction is complete (Fig. 2a,b). Likewise, there is no separability of the environmental effects (Fig. 3b) on MS media. But, the behaviour of the calli changed, regarding the G-response (Fig. 3a): the effect of G1 can be distinguished from those of the other three genotypes, which themselves show no difference in their effects. None of the three can be said to be consistently different from each other.

The most striking result was, that the hormone concentration had no influence on the regeneration capacity of the cultures. Similarly, the environmental factor temperature was expected to be more incisive. The graph of the E-responses (see Fig. 1b) shows that the environments 13, 14, 15 and 16 (the 30 °C temperature curves) range mostly in the upper part of the graph, indicating that growth is slightly increased with increasing temperatures. But nevertheless, the 30 °C effect is undefinable and undistinguishable from all other environmental response functions.

The results of my experiments demonstrate clearly that the genotype effect can be expressed or masked by the applied environ-

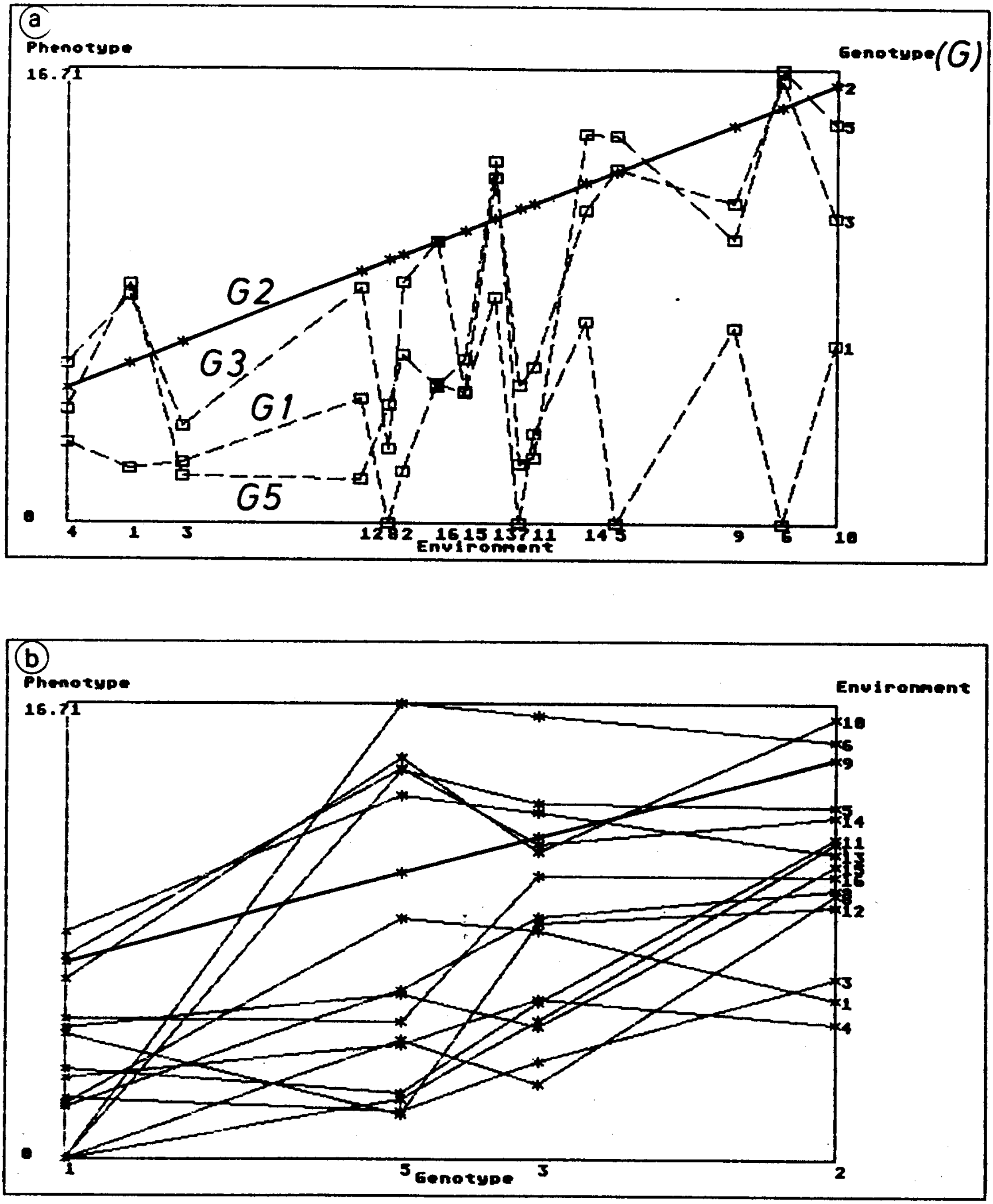

Fig. 1 Callus growth on MS and SO media and four light-temperature conditions. (a) G-response; one-sided separability of the G-effect is to be seen in environments 14 to 10 (MS media). (b) E-response; the response functions are not consistent; interaction is given.

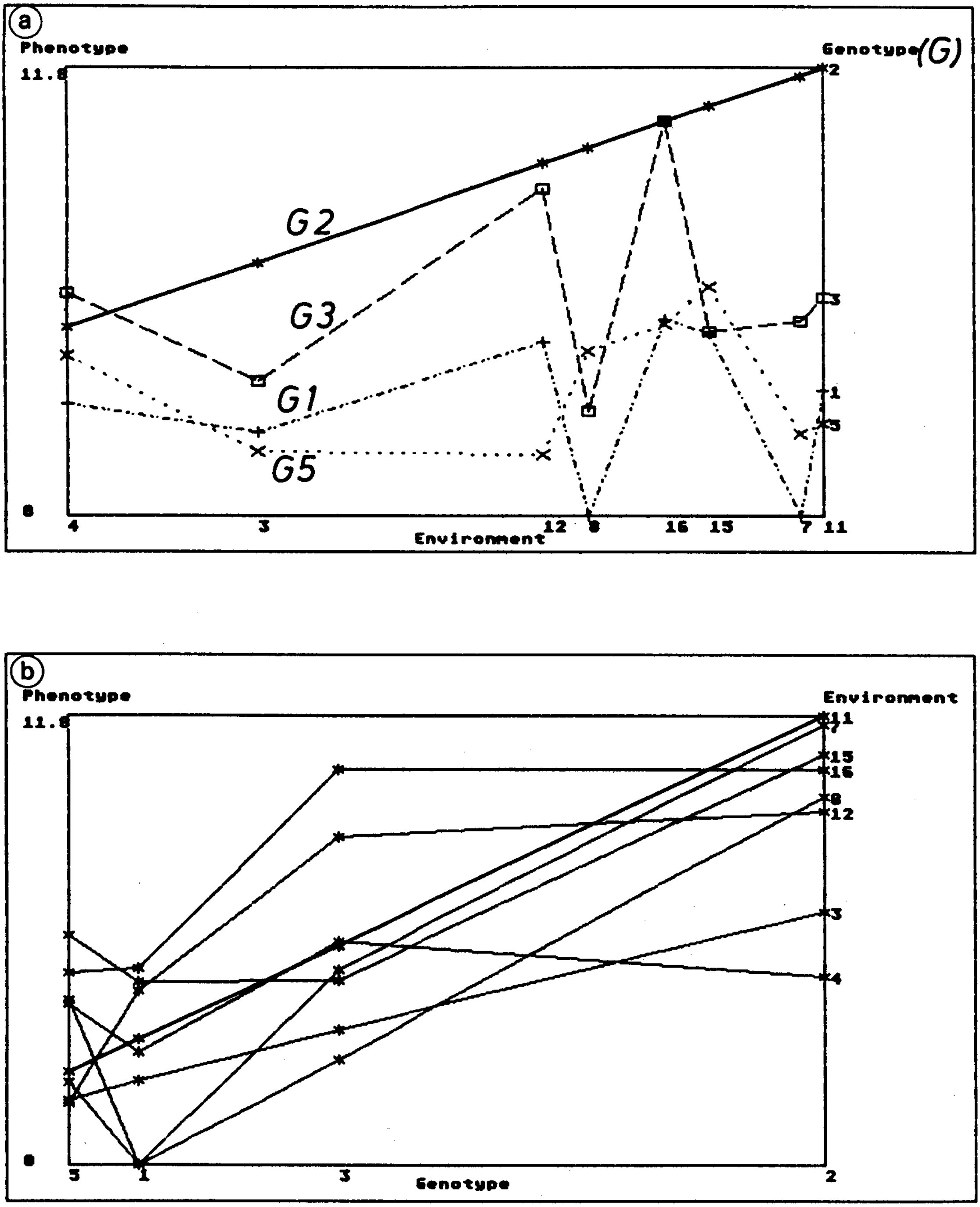

Fig. 2. Callus growth on SO media and four light-temperature conditions. (a) G-response; the effects are not consistent and therefore not separable. (b) E-response; separability is absent. Mutual interaction of both effects takes place.

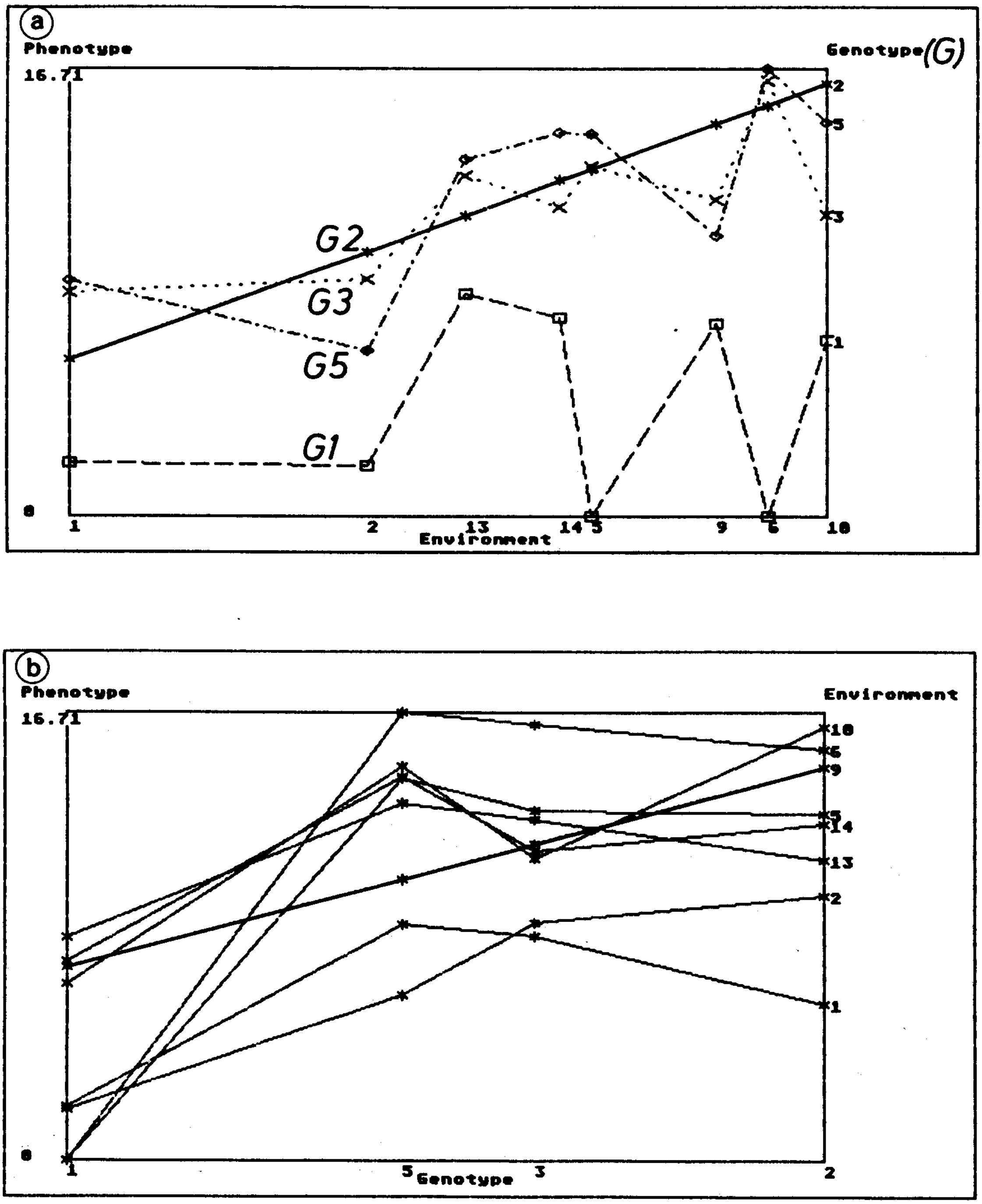

Fig. 3. Callus growth on MS media and four light-temperature conditions. (a) G-response; the effect of G1 is separable. (b) E-response; the functions are not consistent and one-sided interaction takes place.

ments. On MS media the full sibs from self-pollination react in a different way as compared to the non-related genotype. On the contrary, the effect of the Sommer media interacts completely with the effect of all four genotypes; they are not definable and an unequivocal assignment of the effects and their causes cannot be explained.

This example further reveals the mutual influence of both causes. On SO media interaction of both effects is complete; neither G- nor E-effects are consistent and therefore not definable. In other environmental conditions (for example on MS-media) the G-effects gain some preponderence and become detectable. As expected, the full-sibs from self-pollination are identical in their effects because of their close relationship and therefore have a greater probability for alleles identical by descent. They are, however, consistently different from the non-related G1. Here, the separable effect of the cause no. 1, the genetic composition, could be demonstrated. But the effect of cause no. 2, the environmental situation, is interacting with the genotypic effect. Therefore we can confirm mutual interaction in the case of SO-media, but one-sided interaction in the case of MS-media. The results shown here confirm earlier observations of Glock and Gregorius [2] that the genotype has a slightly stronger influence on the character than the environment.

My results allow some more general considerations about separability and interaction. Both are attributes of effects which themselves are produced by causes. The properties of the effects (separability and interaction) can only be valid for a certain group of genotypes in a defined range of environments. For doing tissue culture work the discovery of interaction or separability of one or both effects may have some importance. The one-sided interaction of the environmental effect with that of the genotype can be helpful during mass propagation. The culture conditions seem to be insignificant in a larger range. A mutual separability of both effects gives a hint that these genotypes need very special conditions for growth or regeneration. Further on, the observation of both the effects apart from one another can help to find an optimal environment for one or a group of genotypes. In such a case one would have to look at the environmental response function which ranks consistently above the others, i.e. only one-sided separability of the environmental effects is desired, and it is indifferent, whether the genotypic effects are interacting.

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