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Direct Evidence for Models of Heterosis Provided by Mutants of *Arabidopsis* Blocked in the Thiamine Pathway*[1]

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Summary. Auxotrophic mutants genetically blocked at different steps of the thiamine pathway dramatically demonstrate the biochemical mechanism of hybrid vigor due to simple and perfect dominance at two unlinked loci. Heteroallelic hybrids of mutants requiring the pyrimidine moiety of thiamine display allelic complementation and thus furnish clear biochemical and genetic evidence for the superdominance hypothesis. Hybrids of low- and high-temperature-requiring leaky mutants demonstrate that heterozygosity at a single gene locus may confer developmental homeostasis on the heteroallelic combinations superior to that of the homoallelic parents. The results of this study on the autogamous plant, *Arabidopsis*, and of recent reports on the outbreeding species, *Drosophila*, render untenable the generalization that high versus low temperature dependent heterosis is determined by the breeding system.

Introduction

The several interpretations of heterosis are based on four nuclear mechanisms: additive gene action, dominance, superdominance and epistasis. However, the most recent textbooks of plant breeding in one way or another share the pessimistic view that "despite extensive experimentation over more than forty years conclusive evidence favoring one or the other of these hypotheses has not been forthcoming" (ALLARD 1960, p. 227).

Since the literature on the mechanisms of heterosis is overwhelming, the lack of critical evidence is certainly not the consequence of a lack of interest in the subject. Instead, the inadequacy of the experimental approaches should be blamed. Though statistical methods are powerful tools in modern biology, it is unlikely that basic mechanisms and causal relationships can be revealed by such techniques alone.

The availability of auxotrophic mutants in *Arabidopsis* makes a direct physiological approach to heterosis possible and the experiments provide direct evidence on the dominance and superdominance models of hybrid vigor and the operation of physiological homeostasis at the allelic level.

Material and Methods

In the short life cycle of the autogamous crucifer, *Arabidopsis thaliana*, thiamine (vitamin B_1) auxotrophs can be readily induced by ionizing radiation or by chemical mutagens. The methods for inducing and isolating mutants have been described earlier in detail sufficient for reproducibility (RÉDEI 1965; RÉDEI and LI 1969). The mutants used for these experiments were genetically mapped in three linkage groups (RÉDEI 1965b; LEE-CHEN and STEINITZ-SEARS 1967; LI 1968).

To prevent deficiency symptoms, the mutants were propagated in soil cultures sprayed with thiamine solution (2–5 mg/l) as often as necessary. Crossing did not pose any problems when a 2–5 power magnifier was used. Bagging was not necessary since the chance of an unwanted cross fertilization is in the 10^{-4} range under greenhouse conditions. Since homozygous auxotrophs are lethal types in the absence of the proper supplement, emasculation was not absolutely mandatory in non-allelic crosses. However, during the course of the investigations the anthers of the female parent were always removed before dehiscence with a pair of sharp forceps. With experience thousands of hybrid seeds can be produced daily by one investigator.

The comparative experiments were performed in test tubes on chemically well-defined culture media containing mineral salts, agar and glucose under aseptic conditions (RÉDEI 1965a). All critical experiments were cultured under artificial illumination (ca. 450 footcandle) and controlled temperature as specified in the text.

The performance of the parents and hybrids was evaluated by determination of fresh and dry weight, by measurement of plant height, by estimation of the number of buds, flower and fruits, and by analysis of leaf pigment, protein and thiamine content according to the procedures stated elsewhere (RÉDEI and LI 1969).

Results

Intergenic crosses: The role of dominance in hybrid vigor can be demonstrated dramatically by crossing non-allelic conditional lethals.

Since thiamine is synthesized through various steps of coupling from a special pyrimidine and a special thiazole molecule, several intergenic combinations may be chosen for the demonstration of the dominance hypothesis from the mutants available at four loci. A typical result of a non-allelic cross is illustrated by the hybrids of the mutants which require thiazole (*tz*) and pyrimidine (*py*) (Figure 1). Similarly, perfect intergenic complementation results when mutants of the *py* locus are crossed with mutants of either of the two thiamine loci (*th-1* and *th-2*), or when the *tz* alleles are made heterozygous with mutants of any other loci in this system.

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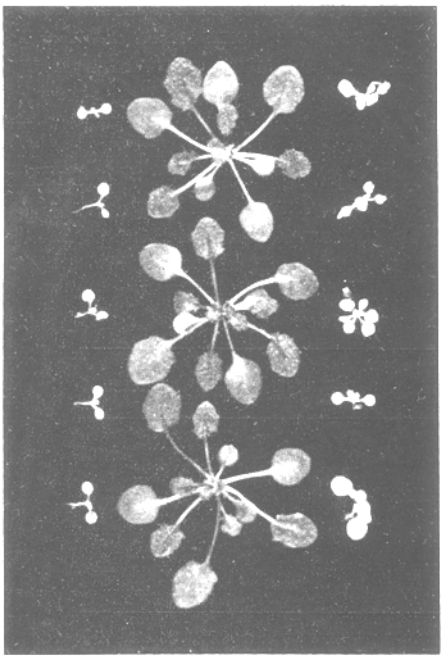


Fig. 1. Intergenic complementation demonstrating the dominance model of heterosis. Left: parent tz^2/tz^2, py^+/py^+; middle: hybrid $tz^2, py^+/tz^+, py^{15}$; right: parent tz^+/tz^+, py^{15}/py^{15}. All genotypes were cultured on glucose-mineral-agar medium in aseptic test-tubes at 15° C for 40 days

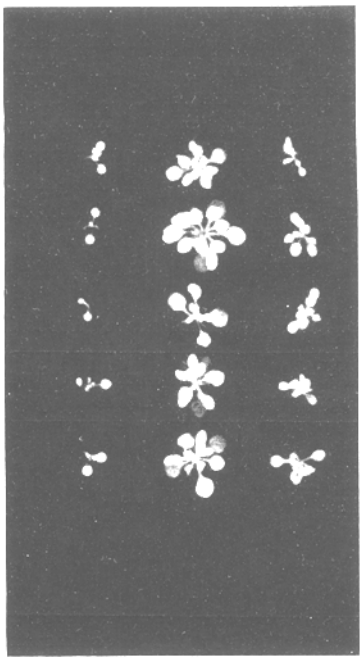


Fig. 2. Allelic complementation at the pyrimidine locus demonstrating the superdominance model of heterosis. Left: parent py^{4ts}; middle: hybrid $py^{4ts} \times py^{5ts}$; right: parent py^{5ts}/py^{5ts}. Culture conditions are the same as indicated in Fig. 1

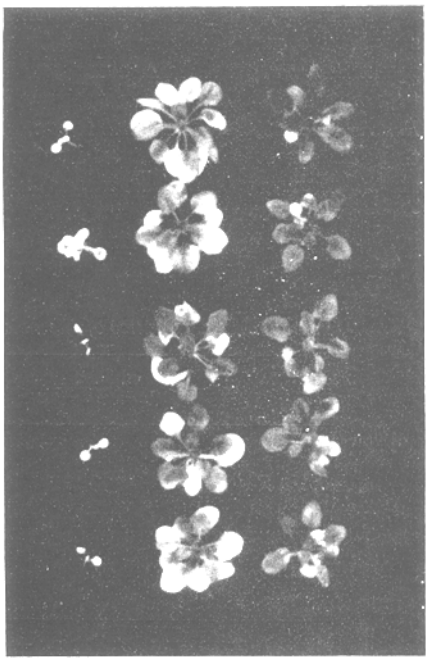
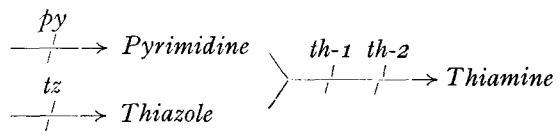


Fig. 3. Allelic complementation at the pyrimidine locus demonstrating the superdominance model of heterosis. Left: parent py^{4ts}/py^{4ts}; middle: hybrid $py^{4ts} \times py^{3ts}$; right: parent py^{3ts}/py^{3ts}. Culture conditions are the same as indicated in Fig. 1

The involvement of only two loci in the heterosis can be easily ascertained. The mutants have been induced on practically perfect isogenic background; *Arabidopsis* is an obligate self-fertilizer with very high genetic stability. The growth and development of all the mutants used in these experiments cannot be distinguished from that of the wild type if the specific requirement is satisfied with a single reagent-grade chemical. The *py* alleles grow perfectly well if 2,5-dimethyl-4-aminopyrimidine or thiamine is supplied in sufficient amount, while the *tz* mutants require 4-methyl-5-beta-hydroxyethyl-thiazole or thiamine. The mutants of the two thiamine loci (*th-1*, *th-2*) respond to the intact thiamine molecule, and neither the pyrimidine nor the thiazole moieties nor a combination of the two are sufficient for normal growth; similarly the *py* mutants cannot utilize thiazole and the *tz* mutants do not respond in any way to the pyrimidine moiety. This behavior of the mutants is understandable and predictable from the synthetic pathway of thiamine and the position of the genetic blocks in that system represented by the gene loci indicated in the following simplified sketch:



Intragenic crosses: With the aid of the 27 independently obtained pyrimidine-requiring mutants the problem of allelic complementation or, by other terminology, "superdominance" has also been investigated. Three of the *py* alleles are of special interest because of their temperature sensitivity. Depending on the nature of the individually different temperature requirement, the plants may grow to some extent even without any supplement. Plants homozygous for the py^{3ts} allele are early seedling lethal at high temperatures; in contrast, under low temperature, some individuals of the same genotype may produce some flowers; the behavior of py^{5ts} homozygotes is the opposite relative to the temperature. The temperature response of the py^{4ts} mutants qualitatively resembles that of py^{5ts}, although the two are easily distinguishable quantitatively. All these mutants are completely recessive to the wild type and all parental genotypes display wild type like growth if the proper pyrimidine is made available, regardless of the temperature within the test conditions (28° and 15 °C).

Two combinations (F_1) of these three alleles display some degree of complementation. The hybrids generally surpass either of the parents at both low and high temperature in weight, height, leaf pigment, protein and thiamine production (Table 1, Figure 2 and Figure 3).

Obviously, we are dealing here with an allelic series. No modifier genes are involved outside the pyrimidine locus. The complementation observed, therefore, satisfies the criteria of superdominance. Alleles of one and only one locus display superiority to both homoallelic parental lines in the heteroallelic state. Numerous combinations of the other 24 *py* alleles fail to show detectable complementation, indicating that the „combining ability" within the locus is quite specific.

in homo- and heteroallelic descendences also reflected the presence of a heterotic component in the F_2. A heritability estimate of 0.59 for the fresh weight was obtained in both hybrids.

Several authors have suggested that genetic and developmental homeostasis plays an important role in the manifestation of hybrid vigor (cf. Lerner 1954; Langridge 1962). Our material appears favorable for the study of developmental homeostasis in case of heterozygosity at a single locus.

Table 1. *Performance of the heteroallelic F_1 combinations relative to the parent (= 100) at 28 °C and 15 °C*

Hybrids	Height	Fresh weight	Flowers and fruits	Leaf pigment	Protein	Thiamine
Cultured at 28 °C						
$py^{4ts} \times py^{3ts}$	198***	131**	139	117	117**	90
$py^{4ts} \times py^{5ts}$	221***	164***	254***	113	122*	134*
Cultured at 15°C						
$py^{4ts} \times py^{3ts}$	94	128*	139	73	98	108
$py^{4ts} \times py^{5ts}$	208***	519***	±	174**	700***	±

No measurements available. — ***, **, * signify difference from the better parent at the .001, .01 and .05 probability level, respectively

The combination $py^{4ts} \times py^{3ts}$ is compared to the py^{4ts} homozygotes at high temperature and to the py^{3ts} parent at low temperature. The reason is that the two parental lines have opposite temperature response and heterosis is expressed as yield relative to the better parent rather than to the less informative mid-parent value. The performance of the $py^{4ts} \times py^{5ts}$ cross is expressed as a percentage of the py^{5ts} parent at both temperatures because the male parent is superior to the female partner under both temperature regimes. The heterosis varies according to the genotypes, growing conditions and characters measured, but the overall eminence of the hybrid is obvious (Table 1).

The selfed F_1 progenies were compared again in F_2 to the two parental lines. The contribution of the heterozygotes to the population means was clearly indicated by respective increases over the homozygotes. A comparison of the frequency distribution

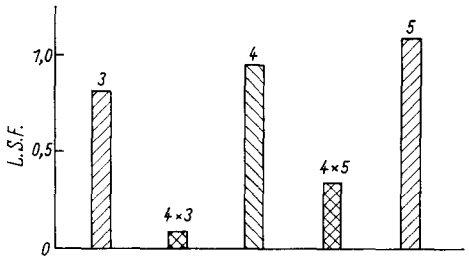


Fig. 4. Developmental homeostasis of parents and intragenic hybrids. Logarithmic stability factor (L.S.F.) was calculated as described in the text. Symbols: 3 = py^{3ts}/py^{3ts}, 4 = py^{4ts}/py^{4ts}, 5 = py^{5ts}/py^{5ts}, 4 × 3 = $py^{4ts} \times py^{3ts}$, 4 × 5 = $py^{4ts} \times py^{5ts}$. In case of maximum stability L.S.F. = 0

In order to quantitate developmental homeostasis Lewis (1954) introduced the concept of a "stability factor". This factor for each genotype was obtained from the ratio of the quantitative expression of the same parameter under two different conditions. If the stability factor, (S.F.) is equal to one, the developmental homeostasis is perfect. Adoption of the original calculational procedure of Lewis creates some problems if populations with opposite responses to environment (as in our study) have to be characterized. If the stability is expressed as the absolute difference between two logarithmic mean values obtained under two different culture conditions, we obtain the logarithmic stability factor (L.S.F.). This L.S.F. has an unequivocal meaning even if the genotypes under study are completely different. If L.S.F. = 0, the developmental homeostasis is maximal. The very effective homeostatic properties of the heteroallelic combinations are made strikingly evident when the L.S.F. method is applied to the fresh weight data of single locus hybrid and parental populations raised under 28° and 15 °C temperature.

Discussion

Heterosis has been the subject of a large number of quantitative genetic studies. Conflicting conclusions concerning the gene action involved have often been drawn from similar experimental material even by the same authors (cf. Moll, Kojima and Robinson 1962).

A few years ago careful and direct genetic analysis made in our laboratory showed that superdominance was the most likely basis of the hybrid vigor experi-

mentally studied in two crosses involving independent genetic factors. The physiological mechanism concerned remained unknown, however, since in those crosses the altered metabolism leading to altered morphology was not amenable to direct analysis (RÉDEI 1962).

It is simple and very revealing to demonstrate hybrid vigor in crosses of auxotrophic mutants in diploids, as the present study shows. The contribution of genes other than the selected ones can be safely ruled out in this study by the sensitive scanning of the chemically well-defined culture media. All the mutants used in these experiments produced perfect phenocopies of the wild type upon the addition of single reagent grade substances to the nutrient solution. Therefore, the genetic material of the parents must not have been affected by the original mutagenic treatment in any unexpected way. The new information thus fully confirms the possibility of superdominance postulated earlier on the basis of genetic analysis alone (RÉDEI 1962).

Studies of this auxotrophic material (RÉDEI and LI 1969) and of a variety of microbial systems (cf. FINCHAM 1966) indicate that in allelic hybrids the condition of partial restoration of function depends on non-identical cistrons which are generally not expected to display full complementation. The question arises then whether or not allelic complementation should be considered as evidence of pseudoallelism rather than of superdominance as suggested by PONTECORVO (1955) and LEWIS (1955). Are we dealing with a problem of semantics or of biology?

For all purposes the best definition of the gene is given by FINCHAM (1966) who equates it with the cistron and considers alleles as mutations in the same cistron.

Biochemical studies tell us that proteins are generally made up of two or more polypeptide subunits. Some enzymes contain a multiplicity of apparently identical polypeptide chains (REITHEL 1963, FINCHAM 1966). A single such subunit is incapable of enzymatic function but—as far as present evidence proves—a dimer made of two identical polypeptide chains constitutes the alkaline phosphatase of *E. coli* (ROTHMAN and BYRNE 1963). Genetic studies indicate that the two subunits of alkaline phosphatase are coded in one cistron (GAREN and GAREN 1963). Genetic and biochemical evidence shows that enzymatically active protein can be formed from certain defective monomers extracted from mutant bacteria and highly purified (SCHLESINGER and LEVINTHAL 1963; FAN, SCHLESINGER, TORRIANI, BARRETT and LEVINTHAL 1966). These results indicate intracistronic complementation.

Enzymatic studies on glutamic dehydrogenase have shown that polypeptide aggregates of different multiplicity are capable of different functions. The 1,000,000 molecular weight crystalline liver enzyme is concerned primarily with glutamate; the 250,000 molecular weight subunits have preferential activity for alanine while the 30,000—60,000 M.W. mono- or dimers do not appear active enzymatically (TOMKINS, YIELDING and CURRAN 1961).

Coenzymes like thiamine pyrophosphate may function in conjunction with several proteins. Therefore, pleiotropic effects are expected in thiamine deficiency and pleiotropy is evident from the observations (Table 1).

Thus, diversification and complementary activity due to different alterations in a single functional genetic unit also seems probable on theoretical grounds.

The data obtained in the course of this study lends support to the views that "heterozygotes are better canalized, better buffered in their developmental processes than homozygotes" (LERNER 1954). Direct evidence is now available that temperature-sensitive alleles at a single gene locus may cooperate heterotically and display developmental homeostasis superior to that of both homoallelic parental types. The heterosis is equally clear at high and low temperatures. The facts thus contradict the generalization of LANGRIDGE (1962) that in autogamous species (*Arabidopsis*) heterosis, caused by temperature-sensitive genes, is manifested only at high temperature and not at low temperature. Our study thus supports the views of VAN VALEN (1967) and SPIESS (1967) who have pointed out that heterosis in *Drosophila* occurs within a wide range of temperature. Actually LANGRIDGE's conclusions (loc. cit. p. 23) concerning *Drosophila*, an outbreeding species, are not as rigid as the critics, VAN VALEN and SPIESS, have interpreted them.

Zusammenfassung

Auxotrophe *Arabidopsis*-Mutanten, bei denen verschiedene Schritte der Thiaminsynthese genetisch blockiert sind, lassen deutlich den biochemischen Mechanismus der Heterosis erkennen, der auf einfacher und völliger Dominanz in zwei ungekoppelten Loci beruht. Heteroallele Hybriden von Mutanten, die den Pyrimidinanteil des Thiamins benötigen, zeigen allele Komplementation und liefern damit den klaren biochemischen und genetischen Beweis für die Superdominanz-Hypothese. Hybriden von leaky-Mutanten, die einen niedrigen bzw. hohen Temperaturbedarf haben, zeigen, daß Heterozygotie in einem einzelnen Genlocus den heteroallelen Kombinationen eine Entwicklungshomeostasie verleihen kann, die größer als die der homoallelen Eltern ist. Die Ergebnisse unserer Untersuchungen an der autogamen Pflanze *Arabidopsis* und neuere Ergebnisse bei *Drosophila* lassen die Verallgemeinerung, daß die Art der Temperaturabhängigkeit der Heterosis durch das Zuchtsystem bestimmt wird, nicht zu.

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