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21 December 1965

Heterosis: Complementation by Mitochondria

Abstract. *Many (but not all) hybrids between two genetic pure lines show heterosis; that is the hybrids grow more rapidly or larger than the parental pure lines. The mechanism is not known. Two corn crosses were studied. In one hybrid known to exhibit heterosis, the mitochondria and an artificial 1:1 mixture of parent mitochondria showed heterosis with respect to oxidation and phosphorylation. In the other cross, neither the hybrid plant, its mitochondria, nor the mixture of parent mitochondria showed heterosis.*

Heterosis has been defined as the superiority of a hybrid over its parents in any measurable attribute. Generally, in studies of heterosis, hybrid superiority has been measured by rate of growth and total growth. Heterosis is a product of hybridization, but hybridization need not necessarily result in heterosis. Heterotic growth may be explained by definitive physiological studies of the enzyme and hormonal activities which elicit the initial manifestation of hybrid vigor during the early development of the organism (*1*). Hybrid maize seedlings when compared with their parents germinate earlier, grow faster, and exhibit a higher metabolic activity (*2*). Hanson and co-workers have pointed out that heterosis exhibited by hybrid maize seedlings may be judged by mitochondrial activity (*3*).

No operational descriptions of heterosis have yet been advanced. We now report an operational basis for seedling heterosis with regard to mitochondrial behavior.

Mitochondria were isolated from scutella (diploid cotyledons) or embryonic axes of 2- to 5-day-old maize inbred (Wf9, C103, Ohio 43, Ohio 45) and hybrid (Wf9/C103, Ohio 43/Ohio 45) seedlings grown at 27°C on moist paper toweling. Isolation procedures (4°C) have been described (*4*). Mitochondrial activity was measured at 27°C in a Warburg respirometer by manometric techniques (*5*). The reaction mixture was composed of 40 μmole of α-ketoglutarate, 1.5 mg of adenosine monophosphate, 0.5 mg of thiamine pyrophosphate, 0.33 mg of diphosphopyridine nucleotide, 0.1 mg of cytochrome *c*, 0.1 mg of coenzyme A, 1.5 mg of hexokinase, 0.11 μmole of glucose, 250 μmole of sucrose, 2.5 μmole of $MgSO_4$, and 25 μmole of potassium phosphate in a final volume of 2.5 ml, *p*H 6.8. In general, reactions were started within 90 minutes after the excised tissues were homogenized. Phosphorylation was determined over a 20-minute period (*6*). All measurements are expressed on the basis of mitochondrial nitrogen (*7*).

It is evident (Fig. 1) that mitochondria of the hybrid Wf9/C103 exhibit definite superiority with respect to oxidation. This superiority is manifested regardless of the tissue from which the mitochondria were isolated. The difference in activity (hybrid mitochondria compared with parental mitochondria) is statistically significant (Table 1).

The results with mitochondria from another hybrid and its parents are shown in Fig. 2. In this instance the hybrid is not different from its parents with regard to oxygen uptake. Of utmost importance in the problem of heterosis with regard to oxidative activity is that hybrid Wf9/C103 (Fig. 1) is an extremely heterotic hybrid, heterosis being expressed not only by germination rate and postgermination development, but by height and yield of grain at maturity. Hybrid Ohio 43/Ohio 45, on the other hand, is almost completely nonheterotic, even at maturity, and frequently is indistinguishable from it parents (*2*). The nonheterotic behavior is exemplified by data in Fig. 2, and the statistical analysis in Table 1 shows that this hybrid is not different from its parents.

Mitochondria from the heterotic hybrid have a lower ratio of phosphorus to oxygen (μmole Pi esterified per μatom of O) than did the parental mitochondria but, significantly, phosphorylation and oxidation were markedly greater in the hybrid mitochondria than in the parent mitochondria. While hybrid Wf9/C103 does not exhibit marked superiority in phosphorylation to both parents, we have clear indication of superior phosphorylation by mitochondria from heterotic hybrids (*8*). The oxidative phosphorylation by mitochondria from the nonheterotic hybrid Ohio 43/Ohio 45 again did not differ from that of its parents.

Thus heterosis is reflected in activities of mitochondria prepared from a heterotic hybrid. Similar behavior was observed in mitochondrial preparations from other heterotic hybrids. Hybrids of diverse genetic background exhibited

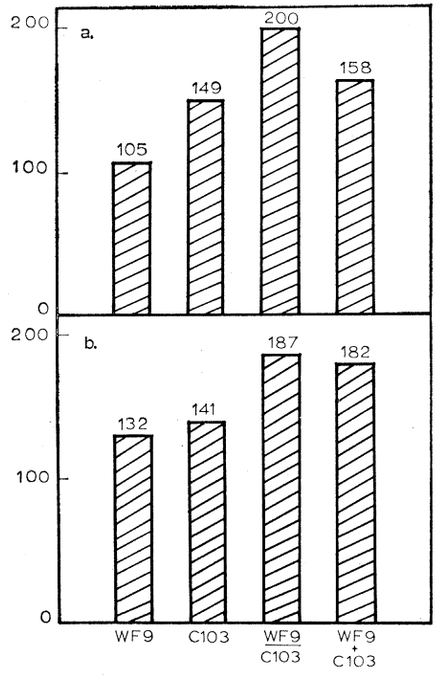

Fig. 1. Oxygen uptake (ordinate, microliters of oxygen per milligram of nitrogen per hour) by mitochondria from maize seedlings. *a*, Mitochondria from 2½-day-old scutella; *b*, mitochondria from 4½-day-old embryonic axes. Wf9 and C103 are inbred strains; Wf9/C103 is hybrid; Wf9 + C103 is a mixture of equal portions of mitochondrial suspension of the inbreds.

Table 1. Duncan's multiple range test. Ranked means (four experiments) of oxygen uptake (microliters per milligram of nitrogen per hour) by mitochondria from 4½-day embryonic axes. Means not underscored by the same line are significantly different at the 5-percent level.

Mitochondrial source:	Wf9	C103	Wf9+C103*	Wf9/C103†
Mean O_2 uptake	132	141	182	187
Mitochondrial source:	Ohio 45	Ohio 43	Ohio 43+ Ohio 45*	Ohio 43/ Ohio 45†
Mean O_2 uptake	136	140	138	144

* 1 : 1 mixture of parent mitochondria. † Hybrid.

Table 2. Oxidative phosphorylation of maize mitochondria prepared from 3-day-old scutella. Data are on the basis of mitochondrial nitrogen.

Strain	O_2 consumed (μatom)	Pi esterified (μmole)	P/O
C103	5.0	10.6	2.12
Wf9	3.2	5.6	1.75
Wf9/C103†	5.9	10.6	1.80
Wf9 + C103*	5.2	14.0	2.69
Ohio 45	3.8	5.0	1.32
Ohio 43	3.4	4.4	1.29
Ohio 43/ Ohio 45†	3.7	5.3	1.43
Ohio 43 + Ohio 45*	3.5	4.3	1.23

* 1 : 1 mixture of parent mitochondria. † Hybrid.

this behavior, suggesting that enhanced mitochondrial activity could be a general biochemical expression of heterosis. The possibility that one of the mechanisms of hybrid superiority is mitochondria-oriented raises questions regarding the genetic transmission of mitochondrial specificity from parent to offspring. Are hybrid mitochondria different from pure line parental mitochondria? With regard to heterotic mitochondrial behavior, are mitochondria from heterotic hybrids different from those of the parents of that hybrid while the mitochondria of the nonheterotic hybrid are *not* different from those of its parents?

Several schemes for mitochondrial origin have been proposed (*9*). For the purpose of this study, we considered the possibilities (i) that mitochondria arise from existing mitochondria as suggested by Luck (*10*) and (ii) that in sexual reproduction the two parental types are passed on gametically to the hybrid. The resulting mixture of the two types of mitochondria could show superior behavior if some form of mitochondrial complementation were to occur in the cells of the hybrid. A test for this hypothesis would be to make mixtures of mitochondria of the parents and to compare the behavior of the mixture with that of the hybrid. It should be emphasized that such a test would establish whether parental mitochondria show any form of complementation. The question as to whether mitochondria are inherited cytoplasmically or by a different process cannot, of course, be resolved by this test.

Mitochondrial mixtures were prepared as follows. Tissues were ground separately; after an initial centrifugation of the homogenate (500*g* for 5 minutes) to sediment cell debris, equal portions of the homogenates were combined and the mitochondria were isolated from this mixture as described (*4*). The mitochondrial pellets were then used, one pellet for each reaction.

The mixture of mitochondria from Wf9 and C103, labeled Wf9 + C103, approaches the behavior of the hybrid mitochondria (Fig. 1). This is especially evident in the experiments with mitochondria isolated from embryos. In the case of scutellar mitochondria, although the behavior of the mixture is somewhat below that of the hybrid mitochondria, the activity as judged by O_2 uptake is still above that of either parent and is not intermediate between the parents as would be expected. The mixture Ohio 45 + Ohio 43 (Fig. 2) shows a complete lack of enhanced activity and, in fact, the O_2 uptake by the mixture is intermediate between the uptake by Ohio 43 and by Ohio 45. The mitochondria thus exhibit in vitro complementation. Mitochondrial mixture Wf9 + C103 is not different in activity from the hybrid mitochondria (Table 1). The mixture of parents of the nonheterotic hybrid, Ohio 43 + Ohio 45, on the other hand is not different from the hybrid mitochondria, but it also is not different from the parental mitochondria. Other systems of inbreds were tested and yielded similar results. It is significant that mitochondria from inbreds which produce a heterotic hybrid undergo complementation. Mitochondria from the parents which do not produce a heterotic hybrid (Ohio 43 and Ohio 45) show no complementation. That the mixing of mitochondria does not lead to some type of uncoupling is shown in line 4 of Table 2, where the mixture Wf9 + C103 shows a high degree of phosphorylation. The mixture Ohio 43 + 45 demonstrates no enhancement with regard to phosphorylation.

Observations of mitochondrial complementation are of interest for several reasons. First, the complementation itself is a striking phenomenon. Second, the pattern of complementation is significant—mitochondria from parents of the heterotic hybrid only undergo complementation. It is unlikely that the complementation is on the substrate level since the reaction contained the necessary cofactors, coenzyme, nucleotide, and the substrate in optimal concentration. Third, the pattern of complementation reflects the phenomenon of heterosis. Fourth, these data indicate that an aspect of heterosis may be studied in vitro on subcellular material. Our data suggest that operational study of the phenomenon of heterosis is a distinct possibility. It is submitted that the feasibility of studying heterosis in vitro would shed a great deal of light on the phenomenon. Admittedly, such work would not elu-

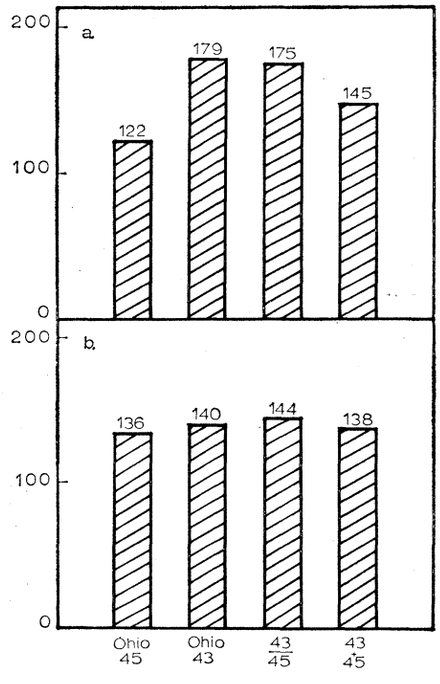

Fig. 2. Oxygen uptake (microliters of oxygen per milligram of nitrogen per hour) by mitochondria from maize seedlings. *a*, Mitochondria from 2½-day-old scutella; *b*, mitochondria from 4½-day-old embryonic axes. Ohio 45 and Ohio 43 are inbred strains; 43/45 is hybrid; 43 + 45 is a mixture of equal portions of mitochondrial suspension of the inbreds.

cidate the precise genetic requirements for heterotic expression. On the other hand, if heterotic phenotype is considered to be superior growth rate, then such studies would reveal the mechanism or mechanisms of accomplishment of this superiority. This knowledge would appear to be equally as important as the knowledge of the genetic factors required for heterosis. Such information may ultimately lead to the elucidation of heterotic genotype (*10*).

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Maturation of a Stress-Activated Mechanism Inhibiting Induction of Tyrosine Transaminase

Abstract. *Rats of various ages were subjected to the stress of 30 minutes on a noisy reciprocating shaker 4 hours before their liver tyrosine transaminase and tryptophan pyrrolase activities were measured. Adrenalectomized infants and adults and hypophysectomized adults were also stressed. Intact, stressed infants exhibited an increase in tyrosine transaminase activity, while intact, stressed adults showed no change. In the stressed adrenalectomized adult, tyrosine transaminase activity markedly decreased, while adrenalectomized infants showed no change. Hypophysectomy largely, but not completely, abolished inhibition in the adults. Tryptophan pyrrolase activity, when present, was increased by stress in all age groups, but the increase was abolished by adrenalectomy and hypophysectomy. The results suggest stress-activation of a pituitary mechanism that inhibits or represses activation of tyrosine transaminase and that may not function during early postnatal life.*

Much evidence indicates that some hormones influence the synthesis of specific enzymes and that synthesis depends on activation of messenger RNA (mRNA). It is indeed possible that such action may underlie a variety of the physiological and biochemical changes produced by these hormones.

The classic work of Knox *et al.* has clearly established that, in the adult rat, exogenously administered adrenal cortical hormones increase the activities of certain liver enzymes participating in protein metabolism (*1*). These enzymes include α-ketoglutarate-tryptophan, tyrosine transaminase, and tryptophan pyrrolase. The effects of stress and of associated cortical hormone secretion on enzyme induction, however, have not been so thoroughly studied.

We have reported (*2*) that adult rats stressed for 30 minutes on a noisy reciprocating shaker did not respond with an increase in tyrosine transaminase activity despite marked adrenalcortical hormone secretion, and that this stress partially inhibited induction by cortisol of transaminase but not of tryptophan pyrrolase activity (*3*).

These observations led us to postulate stress-activation of not only adrenocorticotrophic-hormone (ACTH) secretion but also an additional mechanism (or mechanisms) that inhibited or repressed transaminase induction by the glucocorticoids. Results of additional studies indicated that this repressor mechanism is an adult phenomena that does not function during the early postnatal period (*4*). This report concerns the ontogenesis of this stress-activated inhibitory or repressor mechanism; we present evidence that it is largely abolished by hypophysectomy.

Sprague-Dawley rats bred in this laboratory were used for all experiments but those entailing hypophysectomy (*5*). Littermates of different ages were stressed on a noisy reciprocating shaker (*2, 4*); adrenalectomized and hypophysectomized rats were used 5 to 7 days after surgery. Activities of tyrosine transaminase and tryptophan pyrrolase were determined 4 hours later from portions of liver supernatant (*2*).

Figure 1 indicates that during the first 21 days of life the infant responded to stress with an increase in tyrosine transaminase activity, but that such activity was not increased in similarly stressed intact adults; the results on adults confirm our earlier report (*2*), and the relatively high levels of activity observed during the early postnatal period agree with the observations of Auerbach and Waisman (*6*).

Tryptophan pyrrolase activity was undetectable in either controls or stressed infants before 15 days of age; when it did appear at this time, it showed an increase in response to stress in the different age groups. In 15-day-old infants adrenalectomy prevented the increase in transaminase activity in response to stress, while the adrenalectomized adult, similarly stressed, exhibited a marked decrease in enzyme activity (Table 1). The increase in adult levels of transaminase in controls after adrenalectomy agrees with Knox's data (*1*), although the increase that we report is of a greater order of magnitude.

Adrenalectomy abolished the increase in tryptophan pyrrolase activity in response to stress. Our results thus suggest that in the adult rat this stress, which activates adrenal cortical hormone secretion (*2*), also activates another mechanism that blocks, inhibits, or represses the effects of the glucocorticoids on the induction of tyrosine transaminase, without affecting induction of tryptophan pyrrolase. This conclusion is supported by the observation that in the adult this stress substantially inhibits the transaminase-inducing effects of administration of cortisol (*3*) but not of tryptophan pyrrolase.

The repressor mechanism does not appear to function before 21 days of age, although it is also possible that hepatic tissue is refractory to its influence. The fact that hypophysectomy partially prevents the decrease and that hypophysectomy, together with adrenalectomy, completely prevents it (Table 1) suggests that pituitary factors may contribute to the mechanism. This effect is not an indirect action of ACTH, nor is it caused by adrenalin, noradrenalin, or thyroxine, because adrenalectomized rats exhibited no