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An Evaluation of Mitochondrial Heterosis and in Vitro Mitochondrial Complementation in Wheat, Barley and Maize*

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Summary. Two families each of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) were studied for mitochondrial heterosis and in vitro mitochondrial complementation. Inbred parents and their hybrids were compared for seedling heights and rate of oxygen uptake by the whole tissue to find out if the hybrids showed greater growth and respiratory activity at the seedling stage. Further comparisons were made by isolating mitochondria from the seedling tissues and measuring their ADP:0 ratio, respiratory control ratio and cytochrome c oxidase activity for mitochondrial heterosis. Mixtures of parental mitochondria were similarly compared with parental and hybrid mitochondria for in vitro mitochondrial complementation. No evidence for mitochondrial heterosis or in vitro mitochondrial complementation was found, nor any correlation between the different mitochondrial parameters, seedling heights or rates of oxygen uptake by seedling tissue. The suggested use of mitochondrial heterosis and in vitro mitochondrial complementation for plant breeding is discussed.

Key words: Mitochondrial heterosis – Mitochondrial complementation – *Triticum aestivum* – *Hordeum vulgare* – *Zea mays*

Introduction

Heterosis or hybrid vigor is a genetic phenomenon resulting from heterozygosity, but how heterozygosity leads to heterosis is yet to be explained satisfactorily. In heterotic individuals certain metabolic functions may be expected to be enhanced over those of the inbred parents. Thus the mechanism of heterosis can be investigated at the metabolic level where the gene products are functioning.

With such a hypothesis (Sarkissian 1967), mitochondria from hybrids and their parents were compared by Sarkissian and co-workers (McDaniel and Sarkissian 1966; McDaniel 1967, 1969; Sarkissian and Srivastava 1967, 1969a; Srivastava 1970). They reported 'mitochondrial heterosis', i.e. greater than expected mitochondrial activity in heterotic hybrids, and 'mitochondrial complementation', i.e. enhanced activity in in vitro mixtures of parental mitochondria over the parental averages. Heterosis was considered to be an expression of in vivo complementation between mitochondria inherited from the parents (McDaniel and Sarkissian 1966; Sarkissian and McDaniel 1967). No actual mechanism has been established but McDaniel and Sarkissian (1970) suggested that possibly membrane-mediated changes take place on contact between different mitochondria leading to greater activity. These workers also demonstrated in a maize hybrid a new hybrid-specific mitochondrial type which apparently contributed to the enhanced mitochondrial activity of the hybrid (Sarkissian and McDaniel 1967).

Besides helping to elucidate the phenomenon of heterosis itself, two major possibilities of application have been suggested. First, in vitro mitochondrial complementation may serve as an indicator of the best combining ability, and second, mitochondrial heterosis may be useful in evaluating heterotic hybrids, reducing the need for extensive field trials. These interesting possibilities led to further investigations of mitochondrial heterosis and complementation and their application in predicting hybrid performance in different plant species (Hobson 1971; Doney et al. 1972; Zobl et al. 1972; Sage and Hobson 1973; Ellis et al. 1973). Several mitochondrial activities have been measured, although the emphasis has been on the ADP:0 ratio, which is a measure of the overall efficiency of the mitochondria. There is considerable disagreement among published reports. Ellis et al. (1973) suspected that this could be due to undefined experimental procedures. Hobson (1971) could not repeat the experiments of Sarkissian and Srivastava (1969a) until he introduced modifications in the procedures, but even using Hobson's modifications Ellis et al. (1973) could not duplicate former results.

There is no evidence that mitochondria are transmitted through the male gamete and are multiplied in the offspring. The lack of paternal mitochondria has been demonstrated in maize hybrids (Levings and Pring 1976). This in

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itself can be discouraging for any further efforts to investigate or utilize mitochondrial heterosis and complementation. However, interest in the possibility of utilizing mitochondria for predicting heterosis still exists (see Flavell and Barratt 1977; Barratt and Flavell 1977). In view of such interest, this work is presented in an attempt to clarify the situation.

Several workers at different laboratories had worked on different plant materials with diverse results. Therefore I decided that several plant species should be analysed under the same laboratory conditions, using standardized procedures with the best techniques available. The emphasis was on comparisons of growth, respiratory activity and mitochondrial efficiency at the seedling stage which had been considered to be the ideal stage to investigate the physiology and biochemistry of heterosis (Sarkissian 1967).

Materials and Methods

(i) Plant Materials

Most of the previous work was done on wheat, barley and maize and therefore families with heterotic hybrids were selected from these three species. Mitochondrial heterosis and complementation have been reported from three of these families as noted below. Details are as follows:
Wheat (*Triticum aestivum* L.):
Family A: 31MS, 28, and 31MS X 28 (Sarkissian and Srivastava 1969a, 1970, 1971; Srivastava 1970; Srivastava and Sarkissian 1972)
Family B: A236-A, A236-R, and A236 (= A236-A X A236-R) Srivastava 1970)
Barley (*Hordeum vulgare* L.):
Family A: 63j-7-4, Arivat, 63j-7-4 X Arivat (similar to the one used by McDaniel 1969)
Family B: CG-68-9, Traill, CG-68-9 X Traill
Maize (*Zea mays* L.):
Family A: 601, 303, 601 X 303
Family B: 173D, 203-2, 173D X 203-2

(ii) Methods of Germination

All seeds were surface-sterilized by soaking in an aqueous 0.06% Na-hypochlorite solution for 10 min. followed by thorough washing with running tap water and then distilled water and germinated in the dark at 27°C. Wheat and barley seeds were spread over three layers and covered by one layer of paper towels in covered plastic trays, and maize seeds were planted in vermiculite in small plastic boxes. The media were kept just moist, and no standing water was allowed.

(iii) Measurement of Seedling Heights

For every family, three sets of seeds of each genotype were planted on a particular day, and one set of seedlings were measured on the third, fourth and fifth day after planting. This was repeated four to five different times for each family.

(iv) Measurement of Respiration

Respiration of the whole tissue was measured manometrically using a Gilson differential respirometer. Oxygen uptake by 0.25g of 2.5 day-old shoot tips suspended in 3 ml of water per flask was determined at 27°C. Corrections were made for aqueous vapour pressure and the prevailing atmospheric pressure during every experiment. Oxygen uptake was determined for each genotype on two different days, using two samples per day, and four readings were taken for each sample. Similar tissues were used for the isolation and measurements of activities of mitochondria, except for some measurements on barley as discussed later.

(v) Isolation of Mitochondria

The isolation method was that of Sarkissian and Srivastava (1968) with modifications (Sen 1975). All tissues were harvested just prior to isolation of mitochondria and chilled immediately. When mitochondria were to be used for measuring ADP:0 and R.C. ratios, the grinding buffer contained 0.1% bovine serum albumin (Fraction V, Nutritional Biochemical Corporation). 1:1 mitochondrial mixtures were made after grinding and filtering the tissue and mixing equal volumes of parental tissue homogenates before centrifugation.

(vi) Measurements of Oxidative Phosphorylation

Mitochondrial oxidation rates were determined by an oxygen polarograph (Biological Oxygen Monitor, Model 53; Yellow Springs Instruments Co.) using a Clark type oxygen electrode. ADP:0 and R.C. ratios were determined by the method of Chance and Williams (1955). The terminology used for the different respiratory states was after Chance and Williams (1956). The details of calculations were as elaborated by Sarkissian and Srivastava (1970). The mitochondria were suspended in the same reaction mixture that was used in the reaction well of the oxygen monitor (0.3M mannitol, 0.01M KCl, 0.005M $MgCl_2$, 0.01M KH_2PO_4, 0.01M Tris-HCl buffer, 0.1% bovine serum albumin, pH 7.4). First, the reaction mixture (1.5 ml) was saturated with air and equilibrated for temperature (27°C) in the reaction well by stirring for two min. Then mitochondrial suspension (0.5 ml) containing about 0.5 mg of protein was added and equilibrated for one more min. At the given temperature and on the basis of average atmospheric pressure at the local altitude, the initial O_2 concentration of the saturated mixture was 250 μM. The oxygen electrode was introduced into the well, and 50 μl of 0.612M α-KG (NBC Corp.) was added to give a final concentration of 10mM α-KG. After the respiration stabilized, state 3 respiration was initiated by adding 5 or 10 μl of 30.6mM ADP (Equine muscle, Sigma Chemical) to give a final concentration of 50 or 100 μM respectively. When the mitochondria completed state 3 and respired in state 4 for a measurable period of time, state 3 was initiated again. Four to seven such cycles were measured for each sample of mitochondria. At least two samples were used for each genotype of any family for a single experiment, and at least two experiments were done for each family. To avoid whatever bias may occur due to aging, the assay order of genotypes was reversed between the first and second sample of each day's preparation, and the whole order again was reversed between the two days (one experiment per day).

(vii) Cytochrome c Oxidase Activity Measurements

Mitochondria were suspended in a 20mM K-phosphate buffer (pH 7.4). Cytochrome c oxidase activity was assayed following the

method of Cooperstein and Lazarow (1950) with modifications (Sen 1975). A 25 μM cytochrome c (horse heart cytochrome c, NBC Corp.) solution in 75m M K-phosphate buffer (pH 7.4) was reduced by appropriate amount of 1.2M aqueous solution of Na-hydrosulphite. Ten or 20 μl of mitochondrial suspension (5-20 μg protein) was added to 1.0 ml of the ferrocytochrome c in a cuvette at 25°C and its oxidation was recorded at 550nm. A Hitachi-Coleman 124 double beam spectrophotometer was used with glass-distilled water as reference. In each experiment, four independent assays were made for mitochondria isolated from each genotype of any family. Four experiments were done for each family.

(viii) Protein Determination

Mitochondrial protein was determined by the method of Lowry et al. (1951) using bovine serum albumin (Fraction V, NBC Corp.) as a standard. Absorbance was measured at 660nm with a Spectronic 20 (Bausch and Lomb) colorimenter.

(ix) Analysis of Results

Mean values of all different parameters among the members of each family were compared by F-test. All means were further tested by Duncan's new multiple range test.

Results

Mitochondria of seedlings are generally believed to be most active after about 60 hours of germination (Hanson et al. 1960; Sarkissian and Srivastava 1970; Zobl et al. 1972). Seedling tissue of this age has been used in most of the studies of mitochondrial heterosis and complementation, and the same was done here. If growth rates of plants are results of mitochondrial activities (Sarkissian 1967), they should be manifested in seedlings after they are about two to three days old. Therefore, seedling heights were measured on the third, fourth and fifth days from the time the seeds were soaked.

Seedling height data and their analyses are presented in Table 1. In wheat Family A, only the female parent differed in being shorter throughout the period of measurement. In barley Family A, the female parent started slowly, but by the fifth day there was no difference among the members. In maize Family A, the hybrid was better than the male parent only on the third and fourth days, but the female parent maintained best growth. Distinct growth heterosis for the hybrid at the seedling stage was seen only in

Table 1. Comparison of seedling heights[a]

		Days after planting	Seedling heights (cm)		
Wheat	Family A		31MS	31MS × 28	28
		3rd**	1.37a	1.99b	2.28b
		4th**	3.02a	4.49b	4.97b
		5th**	5.61a	7.89b	8.14b
	Family B		A236-A	A236	A236-R
		3rd	2.02a	1.82a	1.97a
		4th	4.44a	3.86a	4.32a
		5th	7.90a	8.26a	7.27a
Barley	Family A		63j	63j × Arivat	Arivat
		3rd*	3.40a	3.83b	3.99b
		4th	6.22a	7.74b	6.93ab
		5th	10.24a	11.31a	10.46a
	Family B		CG	CG × Traill	Traill
		3rd	4.01a	4.34a	4.19a
		4th	7.32a	7.91a	7.99a
		5th	11.45a	12.44a	12.27a
Maize	Family A		601	601 × 303	303
		3rd**	2.43a	1.45b	0.79c
		4th**	4.46a	4.02a	2.53b
		5th**	8.22a	6.51b	5.26b
	Family B		173-D	173-D × 203-2	203-2
		3rd**	2.58a	4.94b	2.81a
		4th**	4.82a	6.98b	5.15a
		5th**	7.86a	10.58b	9.02a

[a] Values followed by the same letter are not significantly different at 5% level by Duncan's new multiple range test

* F-test shows significant difference among the members at 5% level

** F-test shows significant difference among the members at 1% level

maize Family B where the hybrid showed better growth for all three days.

The data on the oxygen uptake rates are presented in Table 2. In none of the families did the hybrid show oxygen uptake rates that were significantly higher than both parental lines. This was true even for maize Family B, where the hybrid had shown a significantly higher growth rate. Therefore it is noted that not only did the hybrids fail to show general growth heterosis or higher respiration rates at the seedling stage, but also that these two parameters are not always correlated. No methods for measuring and correlating seedling growth and oxygen uptake rates for demonstrating heterosis seem perfect. Criteria for measuring seedling growth may be influenced by factors like nutrient content of the seed or cell elongation unrelated to actual growth. Oxygen uptake would be best compared on per cell or per mitochondrion basis but there is no practicable way of doing so. From this viewpoint. comparing mitochondrial specific activities are much more precise, but relating them to the whole plant is a problem.

ADP:0 and R.C. ratios were obtained from the same materials with the oxygen polarograph. Table 3 shows data on ADP:0 ratios. None of the families, except maize Family A, showed any significant differences between the parents, their hybrids, and the parental mixtures. In maize Family A, the parental mixture differed from the parents, but the hybrid itself, showing ADP:0 ratio closer to that of the parental mixture, did not differ significantly from the inbred parents. Therefore, though this case appeared to be evidence for mitochondrial complementation in vitro, no corresponding mitochondrial heterosis was seen. In this family, the female parent with the lowest ADP:0 ratio had a significantly higher seedling growth and lower respiration rate, whereas the male parent, with second lowest ADP:0 ratio, had lowest growth rate but highest respiration rate.

Data on R.C. ratios are presented in Table 4. No significant differences were found among the members of any family by the F-test. Duncan's new multiple range test showed some differences in one family each of wheat and barley. In neither case was mitochondrial heterosis or complementation indicated. In wheat Family B, the hybrid is significantly lower than the male parent and in barley Family B, the hybrid is significantly lower than the female parent. These two families did not show any differences among the members in their ADP:0 ratios. Maize Family A, which showed differences among its members in their ADP:0 ratios, showed none in the R.C. ratios.

Data on cytochrome c oxidase activities are shown on Table 5. In only barley Family A did one parent (the male)

Table 2. Comparison of oxygen uptake by seedling tissues[a]

		Oxygen uptake rate (ml/h/g of tissue)		
Wheat	Family A	31MS 0.605ab	31MS × 28 0.626a	28 0.506b
	Family B	A236-A 0.753a	A236 0.599a	A236-R 0.527a
Barley	Family A*	63j 0.594a	63j × Arivat 0.461b	Arivat 0.433b
	Family B	CG 0.369a	CG × Traill 0.414a	Traill 0.428a
Maize	Family A*	601 0.551a	601 × 303 0.691ab	303 1.013b
	Family B	173-D 0.726a	173-D × 203-2 0.489a	203-2 0.684a

[a], * For footnotes see Table 1

Table 3. Comparison of ADP:O ratios using α-KG as respiratory substrate[a]

		ADP:O ratio			
Wheat	Family A	31MS 2.283a	31MS × 28 2.281a	28 2.408a	31MS + 28 2.388a
	Family B	A236-A 2.054a	A236 2.055a	A236-R 2.160a	A236-A + A236-R 2.088a
Barley	Family A	63j 1.473a	63j × Arivat 1.680a	Arivat 1.745a	63j + Arivat 1.583a
	Family B	CG 1.973a	CG × Traill 1.654a	Traill 1.700a	CG + Traill 1.913a
Maize	Family A*	601 2.183a	601 × 303 2.375ab	303 2.188a	601 + 303 2.522b
	Family B	173-D 2.180a	173-D × 203-2 2.039a	203-2 2.108a	173-D + 203-2 2.113a

[a], * For footnotes see Table 1

Table 4. Comparison of respiratory control ratios[a]

		Respiratory control ratio			
Wheat	Family A	31MS	31MS X 28	28	31MS + 28
		2.609a	2.582a	2.432a	2.431a
	Family B	A236-A	A236	A236-R	A236-A + A236-R
		1.912ab	1.894a	2.174b	2.049ab
Barley	Family A	63j	63j X Arivat	Arivat	63j + Arivat
		1.680a	1.781a	1.805a	1.691a
	Family B	CG	CG X Traill	Traill	CG + Traill
		1.910a	1.615b	1.720ab	1.698b
Maize	Family A	601	601 X 303	303	601 + 303
		2.523a	2.785a	3.021a	3.341a
	Family B	173-D	173-D X 203-2	203-2	173-D + 203-2
		2.160a	2.068a	1.941a	2.119a

[a] For footnote see Table 1

Table 5. Comparison of cytochrome c oxidase activity[a]

		Cytochrome c oxidase activity (ΔOD_{550}/min/mg protein)			
Wheat	Family A	31MS	31MS X 28	28	31MS +28
		10.080a	10.088a	12.223a	10.740a
	Family B	A236-A	A236	A236-R	A236-A + A236-R
		8.009a	9.556a	9.566a	9.722a
Barley	Family A	63j	63j X Arivat	Arivat	63j + Arivat
		8.336ab	8.587ab	9.894a	7.563b
	Family B	CG	CG X Traill	Traill	CG + Traill
		8.043a	7.587a	8.597a	8.256a
Maize	Family A	601	601 X 303	303	601 + 303
		8.354a	9.653a	8.809a	7.458a
	Family B	173-D	173-D X 203-2	203-2	173-D + 203-2
		7.477a	8.955a	9.200a	9.070a

[a] For footnote see Table 1

differ significantly from the parental mixture. Again, no evidence for mitochondrial heterosis and complementation was found, and no correlations were observed with the other mitochondrial activities.

Discussion

Several problems are encountered in a survey of reports on mitochondrial heterosis and complementation. Lack of reproductibility of results of one worker by another is common, and some of the experimental techniques adopted seem rather arbitrary. For example, Sage and Hobson (1973) used only the first cycle measurements of ADP-stimulated mitochondrial respiration to determine ADP:O, Barratt and Flavell (1977) used only the second cycle (see Flavell and Barratt 1977 also) while other workers used the means of several cycles. Conclusions drawn from selected values can be misleading. Some problems with methodology are discussed below with improvements introduced in this study.

Procedure for isolation of mitochondria is important. Method 'Z' of Sarkissian and Srivastava (1968) coupled with their procedure to remove starch (Sarkissian and Srivastava 1969b) was found not to be the best. The first high speed spin brought down other organelles with mitochondria and took longer time for deceleration. Turning the tubes 90° around their axes before the second spin, separated starch granules, but it was difficult to resuspend the mitochondrial pellet without disturbing the starch pellet. For this study the contaminating particles were separated by using a slow speed spin first with

considerable saving in time. Details have been discussed elsewhere (Sen 1973). Purer mitochondrial preparations thus obtained were important for determining the specific activity of cytochrome c oxidase which was further improved and standardized (Sen 1975). McDaniel did not use bovine serum albumin in his mitochondrial preparation of maize (McDaniel 1967) or barley (McDaniel 1969), but without bovine serum albumin mitochondria became uncoupled very quickly (Sen 1973). Though Ellis et al. (1973) followed the general procedure of McDaniel (1969), they used bovine serum albumin in their mitochondrial preparation.

McDaniel (1969) reported that barley mitochondria obtained from seedlings up to five days old show fairly good respiratory control, with gradual deterioration from the first day onwards. I could not obtain any coupled mitochondria from barley seedlings older than 1.5 days in spite of using modification in the isolation method following McDaniel (1967; 1969) or Bonner (1967). The reason for this could not be ascertained, and 1.5 day-old barley seedlings were used for oxygraph studies while 2.5 day-old seedlings were used for wheat and maize. It is noteworthy that Ellis et al. (1973) used barley embryonic tissue germinated for only 18 hours.

For very young barley seedlings, mitochondria have often been isolated from the scutellum (or cotyledon) as well as the axis of the embryo (McDaniel 1969; Ellis et al. 1973). Perhaps this was unavoidable because of the small size of the seedlings (embryo). However, in studies with maize (McDaniel 1967; McDaniel and Sarkissian 1966, 1968; Sarkissian and Srivastava 1967) the scutella have been used. I found that 2.5 day-old maize tissue yielded mitochondria with much higher activity than scutella of the same age. It was also considerably easier and quicker to obtain mitochondria from the seedlings because of easier harvesting, grinding and lower starch content compared to the scutella. The merit of using transient tissue like scutella for the purpose of this study also seemed dubious. Therefore, as with wheat and barley, the seedling tissue of maize was used.

Tightly coupled mitochondria, i.e. with high R.C. ratio, are necessary for studying ADP:0 ratio. All previous work showed diverse results regarding R.C. ratio, ADP:0 ratio and their roles in reflecting mitochondrial heterosis and complementation. Mitochondrial heterosis and complementation have been reported from barley, with highest recorded R.C. ratio (up to 18) and ADP:0 ratio of only about 75% of the theoretical maximum (McDaniel 1969), from wheat with 'supramaximal' ADP:0 ratio (up to 150% of theoretical maximum) and R.C. ratio of only about 6 (Sarkissian and Srivastava 1969a, 1970) as well as from sugarbeet with comparatively low R.C. (highest 2.48) and ADP:0 (highest 1.73 with α-KG) ratios (Doney et al. 1972). Mitochondrial heterosis and complementation could not be demonstrated by Ellis et al. (1973) with barley, or by Zobl et al. (1972) with wheat, using mitochondria approaching theoretical ADP:0 ratios and moderate R.C. ratios (3 to 5). McDaniel (1969) stated that a higher degree of mitochondrial complementation is obtained in vitro with partially uncoupled mitochondria though better coupled mitochondria with low degree of complementation mimic the in vivo condition, i.e. mitochondrial heterosis, better. No specific correlation was found in this study as has been described in the Results section.

Much of the previous data were not analyzed statistically or were analyzed incompletely. Doney et al. (1972), Zobl et al. (1972) and Ellis et al. (1973) compared the complementing mixtures statistically with the better parent or the mid-parent value and found little evidence for mitochondrial complementation. Sarkissian and Srivastava (1967, 1969a) have not presented statistical analysis. McDaniel and Sarkissian (1968), McDaniel (1967, 1969) and Srivastava (1970) compared the parents, their hybrids and parental mixtures by using only Duncan's new multiple range test but no F-test. The latter test seems important to determine if there are any significant differences at all among the treatments.

The data presented here did not reveal any mitochondrial heterosis or complementation. A lack of differences among the members, including hybrids, were noted in all families. No pattern was seen for the few significant differences found.

The former demonstration of mitochondrial heterosis and complementation remain somewhat unconvincing. A few questions can also be raised about the basic concept and continued investigations based on it. Dissatisfaction with attempts of genetic explanations of heterosis, which have been considered as 'circular arguments', led to the idea of investigating heterosis at the operational level (Sarkissian 1967, 1972). 'Mitochondrial heterosis' was presented as evidence for metabolic superiority of heterotic hybrids (McDaniel and Sarkissian 1966, 1968), and for investigating the phenomenon, mixtures of parental mitochondria were studied assuming that mitochondria are inherited from both parents. These experiments showed 'mitochondrial complementation' (McDaniel and Sarkissian 1966). Subsequently mitochondrial complementation was reported many times, as noted earlier, and mechanisms of complementation were investigated at the organelle (McDaniel and Sarkissian 1970) and enzyme (Sarkissian and Srivastava 1971) levels. Whereas mitochondrial heterosis can be an expression of general heterosis, it is imperative to demonstrate inheritance of mitochondria from both parents before establishing mitochondrial complementation as its cause. Though a study of physical polymorphism of mitochondria in maize by Sarkissian and McDaniel (1967) indicated inheritance of mitochondria from both parents, the

presence of a new hybrid-specific type indicated that mitochondrial types are not determined only by direct gametic transmission. No other similar results have been reported. More recently it has been clearly shown in maize that hybrids inherit mitochondria only maternally (cytoplasmic), and perhaps this is true for all higher plants (Levings and Pring 1976). Certain other considerations were also overlooked. Species that express heterosis very well may show inbreeding depression equally well. Assuming that mitochondria are inherited from both parents, it would be very difficult to imagine why they would cease to complement or be segregrated by inbreeding.

The relationship of yield heterosis of mature plants and mitochondrial heterosis has been included in some studies. McDaniel (1972) has shown positive correlation between grain yields of barley hybrids and mitochondrial heterosis and complementation, but the evidence was not convincing because both mitochondrial heterosis and complementation data were pooled. Zobl et al. (1972) presented mitochondrial complementation and yield data from several wheat families. They found no significant complementation, and the yield data had no correlation with mitochondrial activities. Doney et al. (1972) isolated mitochondria from the roots of sugarbeet and compared mitochondrial complementation with root yield heterosis. Out of seven hybrids with root yield heterosis, only two had parents that showed significant complementation for ADP:0 ratios, but the data were from two different sets of experiments, using tissue from different stages of growth.

Sage and Hobson (1973) suggested that in vitro mitochondrial complementation may serve as a screen for selecting parental lines for hybrid production in wheat. They found mitochondrial complementation of ADP:0 ratios in three hybrids out of nine, but none was statistically significant. Complementation was correlated with the heterosis for yield, only when the hybrids were planted at low seed densities thereby giving them an additional advantage. Barratt and Flavell (1977) reported similar results. From data of such studies where demonstration of yield heterosis itself was difficult, it is hard to decide whether mitochondrial parameters can be used in the prediction of hybrid performance. Sage and Hobson (1973) suggested that measurement of ADP:0 ratios for such predictions are difficult, and some other parameters like cytochrome c oxidase activity may be used. Measurement of cytochrome c oxidase activity yields more consistent values, and results presented here show that such measurements show greater uniformity of activities among parents and their hybrids. Barratt and Flavell (1977) found no complementation with cytochrome c oxidase activity even when they found complementation with ADP:0 ratios. Is mitochondrial complementation then more an artifact of ADP:0 measurements than a fact? Flavell and Barratt (1977) found correlation between mitochondrial ADP:0 and R.C. ratios with grain yields and concluded that better mitochondrial activities in seedling tissues reflect better seed sources rather than mitochondrial activities causing better seedling performances. Their hypothesis involves extramitochondrial small molecules that influence mitochondrial activities. This indicates nuclear control of heterosis and mitochondrial activities are only some possible ways of measuring heterosis. However, the degree of association between mitochondria and external small molecules will depend on whether or not such small molecules are washed off the mitochondria during isolation, a process whose precision can hardly be controlled, making mitochondrial measurements of heterosis unreliable.

With these theoretical problems and experimental discrepancies in the background, this study was carried out using uniform conditions and some improved techniques to determine if mitochondrial heterosis and complementation can be demonstrated definitely. The results are negative. Only unquestionable demonstrations of mitochondrial heterosis and complementation could be of any use in predicting suitable parents for the production of hybrids with superior performance.

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