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Nuclear Gene Dosage Effects Upon the Expression of Maize Mitochondrial Genes

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ABSTRACT

Each mitochondrion possesses a genome that encodes some of its own components. The nucleus encodes most of the mitochondrial proteins, including the polymerases and factors that regulate the expression of mitochondrial genes. Little is known about the number or location of these nuclear factors. B-A translocations were used to create dosage series for 14 different chromosome arms in maize plants with normal cytoplasm. The presence of one or more regulatory factors on a chromosome arm was indicated when variation of its dosage resulted in the alteration in the amount of a mitochondrial transcript. We used quantitative Northern analysis to assay the transcript levels of three mitochondrially encoded components of the cytochrome c oxidase complex (*cox1*, *cox2*, and *cox3*). Data for a nuclearly encoded component (*cox5b*) and for two mitochondrial genes that are unrelated to cytochrome c oxidase, ATP synthase α-subunit and 18S rRNA, were also determined. Two tissues, embryo and endosperm, were compared and most effects were found to be tissue specific. Significantly, the array of dosage effects upon mitochondrial genes was similar to what had been previously found for nuclear genes. These results support the concept that although mitochondrial genes are prokaryotic in origin, their regulation has been extensively integrated into the eukaryotic cell.

MITOCHONDRIA are the cellular sites for many energy conversion processes. Each mitochondrion possesses a genome that encodes a portion of the components necessary for its own biogenesis and functioning. The nuclear genome codes for most of the mitochondrially localized proteins. Mitochondrial transcript levels appear to be subject to cell-specific regulation (*e.g.*, Young and Hanson 1987; Singh and Brown 1993; Monéger *et al.* 1994; Smart *et al.* 1994; Li *et al.* 1996). Although nuclear genes are thought to control mitochondrial transcript levels by influencing transcription and RNA processing, few such genes have been identified. Mitochondrial transcription is accomplished by nuclearly encoded phage T7-like RNA polymerase and associated transcription factors (Newton *et al.* 1995; Gray and Lang 1998).

Direct evidence of the interaction between nuclear and mitochondrial genes comes from the study of cytoplasmic male sterility (CMS). CMS is an economically important plant trait that facilitates the production of hybrid seed without manual emasculation of plants (reviewed by Hanson and Folkerts 1992; Levings 1993). Mitochondrial CMS genes appear to be novel chimeric DNA sequences whose expression perturbs the development of pollen. Genes involved in restoring fertility (*Rf* genes) are nuclear and often act by reducing the abundance of CMS transcripts (reviewed by Schnable and Wise 1998).

CMS can also result from divergent nuclear-cytoplasmic interactions. For example, a nuclear genome from the A619 maize inbred in combination with *Zea perennis* cytoplasm results in plants that are apparently normal except that they are male sterile (Gracen and Grogan 1974). Most other maize inbred lines possess dominant nuclear genes that restore male fertility (Gracen and Grogan 1974; Laughnan and Gabay-Laughnan 1983). Up to three nonallelic dominant genes have been identified that each independently restores fertility to *Z. perennis* cytoplasm (Newton and Coe 1989).

CMS is not the only effect resulting from divergent nuclear-cytoplasmic interactions. A combination of any teosinte cytoplasm from the section Luxurians with the maize inbred W23 nuclear genome results in plants that are small, pale, and slow growing (Allen *et al.* 1989). This "teosinte-cytoplasm-associated miniature" (TCM) trait also results in reduced kernel size. Two dominant nonallelic nuclear genes have been identified that rectify the TCM trait and result in normal plants (Allen *et al.* 1989).

Plants with divergent nuclear-cytoplasmic combinations have also demonstrated nuclear-mitochondrial interactions at the molecular level. In *Z. perennis* and many maize plants with *Z. perennis* cytoplasms the most abundant *cytochrome c oxidase subunit 2* (*cox2*) transcript is a 1.9-kb mRNA. If the maize nuclear genome possesses the dominant *Modifier of cox2 transcript* (*Mct*) gene, then

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a novel 1.5-kb transcript predominates and the 1.9-kb transcript level is reduced (Cooper *et al.* 1990). The novel transcript is due to the activation of a promoter sequence that had been previously undescribed (Newton *et al.* 1995). Thus, *Mct* is a nuclear factor that affects the size and abundance of *cox2* mRNAs by determining transcription start sites.

The purpose of this study was to investigate the interaction of the nuclear genome with mitochondrial gene expression levels in maize with normal cytoplasm. B-A reciprocal translocations were used to create chromosomal dosage series for fourteen chromosome arms. These chromosomal dosage series were used to determine which chromosome arms, when varied in dosage, affected the level of expression of various mitochondrial genes. The basis of the chromosomal dosage effects is believed to be rate-limiting factors that modify gene expression (Guo and Birchler 1994). The effect could be at the level of transcription or it could involve a post-transcriptional aspect of gene expression, such as RNA stability. The dosage effects of 14 chromosome arms were determined for RNA in both embryo and endosperm tissue of kernels 30 days postpollination.

Previous studies have indicated an extensive array of *trans*-acting dosage effects on nuclear genes (Birchler and Newton 1981; Guo and Birchler 1994). Mitochondrial genomes, having evolved from a prokaryotic progenitor, could differ significantly in regulatory processes, so it was of interest to determine whether the same types of effects were operative on organellar genes. We also examined the degree to which related mitochondrial genes are coordinately affected. Finally, we determined the effects upon the expression level of a nuclearly encoded mitochondrial protein. The effects upon the nuclear gene were compared to the effects upon the mitochondrial genes to determine whether they are subject to the same types of modulations.

MATERIALS AND METHODS

Production and identification of aneuploids: Dosage series were produced using B-A reciprocal translocations. B chromosomes are supernumerary and often nondisjoin at the second pollen mitosis. This results in one sperm of a pollen grain having two B chromosomes and the other having none (Roman 1947). A B-A translocation involves an exchange of part of the B chromosome with one of the 10 chromosomes that make up the haploid set of maize (*i.e.*, an A chromosome). The translocation chromosome with the B centromere and an arm of an A chromosome generally behaves like the B chromosome and regularly nondisjoins at the second pollen mitosis (see Beckett 1978). In that case one sperm has two copies of the translocated A chromosome arm and the other has none. This creates an opportunity to produce aneuploids. Fertilization of the egg with the sperm that possesses two B-A's yields individuals that are hyperploid for the A chromosome arm; fertilization with the deficient sperm results in progeny that are hypoploid for that same chromosome arm. Euploid progeny are also produced because some of the pollen grains possess sperm where the B-A's disjoined normally.

TABLE 1

B-A stocks and testers used to produce dosage series

B-A stock	Marker gene	Tester stock
TB-1Sb	*P1-vv* (*Ac*)	*r1:sc-m3* (*Ds*)
TB-1La	*Bz2*	*bz2*
TB-2Sa	*B1-peru*	*b1; r1*
TB-3La	*A1*	*a1*
TB-4Sa	*Dt6*	*a1-m1* (*rdt*)
TB-4Lb	*C2*	*c2*
TB-5Sc	*A2*	*a2*
TB-5Ld	*Pr*	*pr*
TB-6Lc	*Dt2*	*a1-m1* (*rdt*)
TB-7Lb	*Dt3*	*a1-m1* (*rdt*)
TB-8Lc	*Ac2*	*bz2-m* (*Ds*)
TB-9Sd	*C1*	*c1*
TB-9Lc	*Ac*	*r1:sc-m3* (*Ds*)
TB-10L19	*R1-scm3*	*r1*
TB-10L32	*R1-scm3*	*r1*

B-A stocks are named for the translocation present. B-A chromosomes are marked with either a color factor (*A1*, *A2*, *B1-peru*, *Bz1*, *Bz2*, *C1*, *C2*, *Pr1*, or *R1-scm2*) or an autonomous transposable element (*Ac* or *Dt*). Except for the recessive color factor indicated above, each tester stock was homozygous for *A1*, *A2*, *b1*, *Bz1*, *Bz2*, *C1*, *C2*, *Pr1*, and *R1-scm2*. Several testers had color factors that possessed insertions of a nonautonomous transposable element (*Ds* or *rdt*).

Fifteen different maize stocks, each possessing a different B-A translocation, were used to generate dosage series (Table 1). Each stock is named for the translocation present. Translocations are designated according to which chromosomes are involved. Maize chromosomes are numbered from 1 to 10, each possessing a long (L) and a short (S) arm. TB-1Sb involves a translocation between a B chromosome and the short arm of chromosome 1. Each translocation is also given an alphabetical or numerical suffix so that it can be distinguished from other translocations involving the same chromosomes. For example, we have included both TB-10L19 and TB-10L32. TB-10L19 has all of 10L attached to the B centromere, while TB-10L32 has only the most distal 26% of 10L (Maguire 1985). To simplify our text we omitted the TB prefix because in each case it is understood that what has been manipulated was a translocation with a B chromosome.

Each translocation stock had the B-A chromosome marked with a dominant genetic marker, either an anthocyanin color gene or an autonomous transposable element (Table 1). Plants with marked B-A chromosomes were crossed onto an appropriate tester plant. Testers possessed either a recessive allele of a color marker gene or a reporter gene that produces colored spots in the presence of an autonomous transposable element (Table 1). Reporter genes are color factors that are nonfunctional due to the insertion of a nonautonomous transposable element. In the presence of a related autonomous element, the nonautonomous element sporadically excises from the gene, causing it to become functional.

The marker system allowed for the determination of chromosome arm dosage because nondisjunction of the B-A chromosome resulted in noncorrespondence of embryo and the endosperm phenotypes (Birchler and Alfenito 1993). When the sperm with two B-A's fertilized the egg, the embryo was hyperploid and colored (or spotted). These colored embryos had hypoploid colorless endosperm because the other sperm of the pollen grain had no B-A and this deficient sperm fertil-

TABLE 2

Probes used for Northern analysis

Gene	Description	Clone	Plasmid	Clone source
cox1	Cytochrome c oxidase subunit 1	0.57-kb maize mtDNA	Bluescript SK (Stratagene)	E. Kuzmin (unpublished data)
cox2	Cytochrome c oxidase subunit 2	0.70-kb maize mtDNA	Bluescript KS (Stratagene)	Fox and Leaver (1981)
cox3	Cytochrome c oxidase subunit 3	1.1-kb *Oenothera* mtDNA	Bluescript KS (Stratagene)	Hiesel *et al.* (1987)
cox5b	Cytochrome c oxidase subunit 5b	0.9-kb maize cDNA	pSport1 (GIBCO BRL, Gaithersburg, MD)	Pioneer HiBred Intl. (unpublished data)
atpA	ATP synthase α-subunit	0.33-kb maize mtDNA	Bluescript KS (Stratagene)	Mulligan *et al.* (1988)
rrn18	18S mitochondrial rRNA	1.2-kb maize mtDNA	Bluescript KS (Stratagene)	Mulligan *et al.* (1988)
rRNA	26S nuclear rRNA	0.8-kb maize genomic	Bluescript KS (Stratagene)	Messing *et al.* (1984)

ized the central cell. Alternatively, when the sperm with two B-A's fertilized the central cell, the endosperm was hyperploid and colored and the embryo was hypoploid and colorless. Normal disjunction of the B-A at the second pollen mitosis resulted in both the embryo and endosperm being euploid and having color since each received one copy of the B-A.

Plants were grown and pollinations were made as described by Neuffer (1994). Kernels were harvested 30 days after pollination. Embryos and endosperms were classified, separated, and immediately frozen in liquid nitrogen. Tissues were stored at −80°.

RNA isolation: RNA was extracted from frozen tissue. Typically 10–20 embryos (∼1 g) from the same ear with the same chromosome arm dosage were pooled. Similarly, 5–15 endosperms (∼2 g) were combined. Total RNA was isolated using a method similar to that used by Cone *et al.* (1986). Tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. About 10 ml of lysis buffer was rapidly and vigorously added per gram of frozen ground tissue. The lysis buffer was 2% sodium dodecyl sulfate (SDS), 0.1 m sodium chloride (NaCl), 50 mm Tris-HCl (pH 7.4), 50 mm disodium ethylenediaminetetraacetate (EDTA; pH 8), and 200 μg/ml proteinase K. The supernatant was subjected to three extractions: the first two with phenol (pH 7.9):chloroform (1:1) and the last with pure chloroform. After each extraction the samples were centrifuged at 12,000 × *g* for 10 min. Total RNA was precipitated with LiCl (2 m final solution). The RNA was redissolved and reprecipitated with 2.5 volumes absolute ethanol and 0.1 volume 3 m sodium acetate (pH 5.4). The RNA was then redissolved in formamide and the concentration was determined by measuring the optical density of a sample at 260 nm.

Electrophoresis and blotting: Samples were subjected to electrophoresis in formaldehyde-agarose (1.5%) gels. Each lane was loaded with 5 μg of total RNA. RNA was transferred to uncharged nylon membranes by capillary blotting. The RNA was immobilized on the membranes by UV cross-linking.

Methylene blue staining: Blots were stained with methylene blue (Herrin and Schmidt 1988). They were immersed in 5% acetic acid and gently agitated for 15 min. The acetic acid was replaced with a methylene blue staining solution (0.5 m sodium acetate, pH 5.4; 0.04% methylene blue) and allowed to agitate for another 10 min. Blots were rinsed in deionized distilled water. The moist blots were wrapped in clear plastic and scanned on a conventional flat bed scanner.

Hybridization: Hybridization conditions were essentially the same as in Birchler and Hiebert (1989) for RNA antisense probes. The blots were prehybridized for 4 hr at 60°. A ^{32}P-radiolabeled probe was added and allowed to hybridize for an additional 20 hr at the same temperature. Except for the nuclear 26S rRNA, antisense RNA probes were prepared by *in vitro* transcription using T3, T7, or Sp6 RNA polymerase (Promega, Madison, WI). Probes for rRNA were synthesized by PCR using T3 and T7 primers. The hybridization solution was 50% formamide, 5× SSC (0.75 m NaCl; 0.075 m sodium citrate), 10× Denhardt's solution, 0.5% SDS, 10% dextran sulfate, and 0.2 mg/ml fragmented salmon sperm DNA. The blots were washed for 2 hr at 75° with four changes of wash solution. The wash solution was 0.2× SSC and 0.05% SDS. When a double-stranded DNA probe was used to detect rRNA, the prehybridization and hybridization temperature was 55°, the wash temperature was 65°, and no dextran sulfate was used in the hybridization buffer.

Probe sources: All clones were inserted into plasmids that allowed for *in vitro* transcription (Table 2). The clone for cytochrome c oxidase subunit 1 (*cox1*) was obtained from E. Kuzmin (unpublished results), who cloned a 0.57-kb *Pst*1/*Apa*LI fragment from a maize CMS-S λ-library. This fragment covers the 5′ portion of the *cox1* gene. Part of the original clone for maize *cox2* (Fox and Leaver 1981) was subcloned into pBluescript KS+ by K. Yamato in the K. Newton laboratory. The original clones for cytochrome c oxidase subunit 3 (*cox3*; Hiesel *et al.* 1987) and mitochondrial 18S rRNA (*rrn18*; Mulligan *et al.* 1988) were subcloned into pBluescriptII KS+ by M. Guo in the J. Birchler laboratory. M. Guo also subcloned a 0.8-kb *Xho*I/*Eco*RI fragment of 26S rDNA into pBluescriptII KS+ from an original 9-kb clone (Messing *et al.* 1984). The clone for cytochrome c oxidase subunit Vb (*cox5b*) provided by Pioneer Hi-Bred Intl. (unpublished results) was an expressed sequence tag that was identified by its homology with *cox5b* of rice (Kadowaki *et al.* 1996). The clone for ATP synthase α-subunit (*atpA*; Mulligan *et al.* 1988) was provided in pBluescript KS+.

Data analysis: Hybridized transcripts were quantitated using a Fuji (Stamford, CT) phosphorimager. Nuclear 26S rRNA was used as the gel-loading control. It was previously demonstrated that rRNA is a reliable loading control that shows no demonstrable fluctuation in expression level for the various chromosome arm dosage series (Guo and Birchler 1994). The signal strength of the rRNA was obtained either from the methylene blue image or from subsequently probing the blot with a radiolabeled rRNA probe. The methylene blue images were quantitated using the same Fuji software that was used for the radiolabeled probes. The relative quantity of the probed RNA was determined from the ratio of the mRNA/rRNA signal strength.

For each chromosome arm there were three doses: hypoploid, euploid, and hyperploid. Three samples were made for each tissue at each dosage. Means and standard errors were calculated from the three samples. Because the essential question was how mRNA levels of an aneuploid differ from the

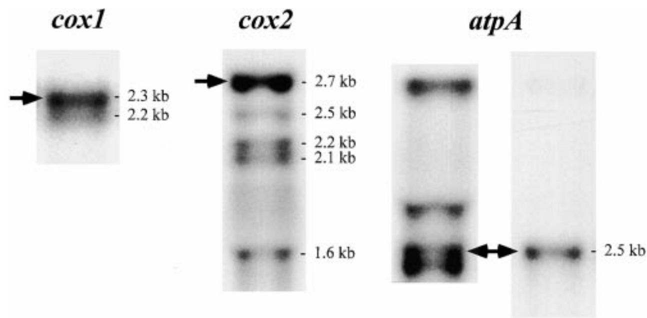

Figure 1.—RNA species with multiple transcripts. Three genes gave multiple transcripts: *cox1*, *cox2*, and *atpA*. Arrows indicate the particular transcripts used in this study. Note that while *atpA* gave multiple transcripts, only the 2.5-kb transcript was reliably represented on all blots. A double-headed arrow indicates this.

euploid, the mean of the mRNA/rRNA value for each dose was divided by the mean of its respective euploid mRNA/rRNA signal. This resulted in all euploids having an expression level of one and any variation in the expression level of the aneuploid was evident as a deviation from one. Significant differences between aneuploid and euploid expression levels were determined by *t*-tests ($P \leq 0.05$).

RESULTS

The expression levels of five mitochondrial genes were assayed. Three of them (*cox1*, *cox2*, and *cox3*) encode subunits of the cytochrome c oxidase complex of the mitochondrial electron transport chain. The two other mitochondrial genes encode proteins involved with the ATP synthase and ribosomal complexes (*atpA* and *rrn18*). We also assayed effects upon a nuclearly encoded component of the cytochrome c oxidase complex (*cox5b*).

Three genes showed a multiple banding pattern (Figure 1). In each case, one band was selected to obtain data for this study. The *cox1* gene produced two transcripts. Our estimated sizes of 2.3 and 2.2 kb were very close to the published transcript sizes of 2.4 and 2.3 kb (Isaac *et al.* 1985a). The data presented in Figures 2 and 3 are for the more abundant larger molecular weight transcript. The *cox2* gene produces multiple transcripts ranging from 2.6 to 0.6 kb (Fox and Leaver 1981). Although as many as 11 have been reported (Lupold *et al.* 1999a), 5 bands showed prominently on our blots. Again, the data presented are for the highest-molecular-weight transcript, estimated on our blots as 2.7 kb in size. It was typically the most intense band. Although *atpA* had multiple bands, only the 2.5-kb band was consistently observed on all blots and it was used for this study. It is interesting to note that Isaac *et al.* (1985b) described a complex banding pattern with three major transcripts (2.5, 3.0, and 4.5 kb), while Braun and Levings (1985) indicated two transcripts, a strong 2.6-kb band and a much weaker 5.0-kb band. Both of those studies used RNA from coleoptiles.

The data for the expression levels of all six genes are presented in Figure 2 for the embryo tissue and Figure 3 for the endosperm tissue. Although each dosage series is composed of three conditions (hypoploid, euploid, and hyperploid), statistical analysis was performed by pairwise comparisons of aneuploid to the euploid. A shaded box indicates aneuploid expression levels that were significantly different from the euploid ($P \leq 0.05$ per *t*-test). In each pairwise comparison, the effect was considered positive if the sample with the higher dosage had an increased expression level, *i.e.*, hypoploid less than euploid or euploid less than hyperploid. The effect was considered negative if the higher ploidy sample had a lower expression level, *i.e.*, hypoploid greater than euploid or euploid greater than hyperploid.

There were eight combinations of effects that resulted from dosage series (Figure 4). In several cases both the hypoploid and hyperploid resulted in effects relative to the euploid that were consistent; *i.e.*, both were positive or both were negative. These are called series effects. The effect of the 5Lb dosage series upon *cox2* in the embryo is shown as an example of a positive series effect; *i.e.*, the transcript level increases with the copy number of 5L. The effect of the 1La dosage series on *cox5b* in the embryo is shown as an example of a negative series effect. More rare were the mixed effects (Figure 4), where the hypoploid and hyperploid effects were opposite from each other. If both aneuploids had transcript levels that were less than the euploid, then the effect of hypoploidy was positive and the effect of hyperploidy was negative. If both aneuploids had transcript levels that were greater than the euploid, then the effect of hypoploidy was negative and the effect of hyperploidy was positive. The two types of mixed effects are exemplified by the pattern of 5Sc on *atpA* and 8Lc on *cox2* in embryo tissue (Figure 4). More common were the cases where the effect occurs either in only the hypoploid or the hyperploid. Four examples, all from endosperm comparisons, are shown in Figure 4 to illustrate these cases.

Dosage effects upon mitochondrial genes: The array of effects upon the mitochondrial genes (Figures 2 and 3, *cox1*, *cox2*, *cox3*, *atpA*, and *rrn18*) were generally similar to what was found in a previous study on chromosomal dosage on nuclearly encoded genes (Guo and Birchler 1994). All chromosome arms had an effect upon one or more gene products but no chromosome arm affected all genes in all tissues. All genes were affected by more than one dosage series; no gene was affected by all dosage series. Each gene was affected by an average of seven dosage series per tissue. A notable variant from this average was *rrn18* with half the number of dosage series that affected its expression level. These results are consistent with the idea that these effects are due to the action of individual factors distributed throughout the genome (Guo and Birchler 1994).

The predominant effect was negative. Forty-two sig-

EMBRYO

B-A	Dose	*cox1*	SE	*cox2*	SE	*cox3*	SE	*atpA*	SE	*rrn18*	SE	*cox5b*	SE
1Sb	hypo	1.02	0.03	0.88	0.04	0.84	0.04	1.03	0.05	1.02	0.01	1.01	0.05
	eup	1.00	0.02	1.00	0.05	1.00	0.02	1.00	0.03	1.00	0.01	1.00	0.08
	hyper	0.98	0.07	1.11	0.07	1.08	0.05	0.94	0.08	0.97	0.02	1.33	0.09
1La	hypo	1.61	0.04	0.96	0.05	0.59	0.02	1.16	0.02	1.27	0.11	1.31	0.08
	eup	1.00	0.03	1.00	0.01	1.00	0.08	1.00	0.05	1.00	0.02	1.00	0.00
	hyper	0.58	0.01	0.99	0.03	1.06	0.05	0.77	0.02	0.89	0.03	0.83	0.01
2Sa	hypo	0.93	0.02	1.13	0.01	0.98	0.08	0.98	0.03	1.02	0.01	0.93	0.05
	eup	1.00	0.01	1.00	0.01	1.00	0.05	1.00	0.04	1.00	0.02	1.00	0.03
	hyper	0.91	0.03	0.98	0.08	0.98	0.03	1.00	0.02	1.00	0.06	1.03	0.02
3La	hypo	1.15	0.08	1.02	0.06	1.46	0.15	1.01	0.02	1.05	0.05	0.98	0.05
	eup	1.00	0.06	1.00	0.07	1.00	0.14	1.00	0.07	1.00	0.03	1.00	0.04
	hyper	1.12	0.05	1.27	0.03	1.02	0.10	1.09	0.12	0.94	0.02	1.53	0.08
4Sa	hypo	1.11	0.17	0.75	0.09	0.80	0.08	1.34	0.16	0.93	0.02	0.79	0.00
	eup	1.00	0.08	1.00	0.06	1.00	0.07	1.00	0.01	1.00	0.04	1.00	0.04
	hyper	0.95	0.10	0.97	0.05	0.76	0.05	1.26	0.09	1.08	0.05	0.69	0.02
4Lb	hypo	1.07	0.04	1.12	0.10	1.05	0.03	1.10	0.05	1.01	0.02	1.00	0.03
	eup	1.00	0.01	1.00	0.08	1.00	0.03	1.00	0.01	1.00	0.02	1.00	0.02
	hyper	1.12	0.03	1.02	0.08	0.98	0.04	0.93	0.03	0.99	0.02	0.95	0.05
5Sc	hypo	1.06	0.02	1.34	0.04	1.05	0.00	0.59	0.01	0.91	0.02	1.03	0.04
	eup	1.00	0.00	1.00	0.06	1.00	0.01	1.00	0.03	1.00	0.03	1.00	0.05
	hyper	1.01	0.08	0.52	0.03	1.03	0.05	0.86	0.04	0.97	0.02	1.06	0.05
5Lb	hypo	1.11	0.12	0.72	0.00	0.92	0.01	0.52	0.08	0.94	0.15	0.96	0.03
	eup	1.00	0.04	1.00	0.01	1.00	0.03	1.00	0.09	1.00	0.07	1.00	0.07
	hyper	1.07	0.10	1.56	0.13	1.03	0.07	0.90	0.07	0.98	0.10	0.84	0.02
6Lc	hypo	1.00	0.05	1.78	0.06	1.40	0.07	1.32	0.06	0.90	0.03	1.36	0.05
	eup	1.00	0.02	1.00	0.04	1.00	0.03	1.00	0.06	1.00	0.04	1.00	0.08
	hyper	1.09	0.05	1.04	0.06	0.77	0.03	0.67	0.05	0.99	0.10	0.65	0.01
7Lb	hypo	0.93	0.04	0.96	0.04	1.06	0.09	0.82	0.07	0.78	0.13	1.08	0.04
	eup	1.00	0.03	1.00	0.05	1.00	0.03	1.00	0.05	1.00	0.11	1.00	0.07
	hyper	1.02	0.01	1.05	0.05	1.00	0.15	0.94	0.04	0.89	0.10	1.01	0.09
8Lc	hypo	1.16	0.08	1.26	0.04	1.13	0.08	1.15	0.11	0.96	0.05	1.04	0.03
	eup	1.00	0.01	1.00	0.01	1.00	0.02	1.00	0.03	1.00	0.02	1.00	0.02
	hyper	1.06	0.03	1.17	0.05	1.03	0.02	1.08	0.13	0.98	0.04	1.01	0.04
9Sd	hypo	1.01	0.05	1.10	0.07	1.04	0.12	1.08	0.03	1.09	0.06	0.93	0.03
	eup	1.00	0.05	1.00	0.06	1.00	0.09	1.00	0.01	1.00	0.05	1.00	0.02
	hyper	0.92	0.05	1.04	0.09	1.12	0.10	1.01	0.02	1.02	0.04	0.92	0.04
9Lc	hypo	1.53	0.05	1.18	0.03	1.08	0.05	0.93	0.04	1.00	0.01	1.00	0.04
	eup	1.00	0.01	1.00	0.03	1.00	0.04	1.00	0.05	1.00	0.02	1.00	0.02
	hyper	1.06	0.03	1.08	0.03	1.16	0.06	1.18	0.10	1.04	0.08	0.90	0.03
10L19	hypo	1.21	0.07	1.06	0.01	1.40	0.01	1.26	0.06	0.98	0.09	0.75	0.04
	eup	1.00	0.04	1.00	0.04	1.00	0.01	1.00	0.04	1.00	0.08	1.00	0.06
	hyper	1.04	0.04	1.02	0.02	1.18	0.09	0.95	0.04	1.00	0.02	1.00	0.06

Figure 2.—The mRNA levels of six genes for 14 dosage series in embryo tissue. Each dosage series was composed of hypoploid (hypo), euploid (eup), and hyperploid (hyper) samples. The mRNA level for each member of a dosage series is shown relative to the euploid level (set at 1.00) along with its standard error (SE). The B-A translocation used to create each dosage series is listed on the left under B-A. Values that are significantly different ($P \leq 0.05$, *t*-test) from the euploid are indicated by shaded boxes.

nificant pairwise effects (*i.e.*, hypoploid *vs.* euploid or euploid *vs.* hyperploid) were detected in the embryo tissue. Of these, 14 were positive and 28 were negative. There were 50 significant pairwise effects detected in the endosperm tissue. Of these, 21 were positive and 29 were negative. For the most part, effects were tissue specific; *i.e.*, similar dosage effects upon a gene were unlikely to be found in both embryo and endosperm. Some exceptions were the effects of 1La and to a lesser extent the effects of 6Lc (Figures 2 and 3). Comparison of the effects of 1La on the mitochondrial genes in embryo *vs.* endosperm yielded only two differences between embryo and endosperm. The dosage effects of 6Lc were less consistent except that, in all cases where effects were detected, they were negative.

Differences in the effects between the 2.3- and 2.2-kb *cox1* transcripts: Although the 2.3-kb *cox1* transcript was selected for this survey, it was apparent that the 2.2-kb band was affected differently in several dosage series. The two *cox1* transcripts result from two transcriptional start sites that are 95 bases apart (Isaac *et al.* 1985a). Unless the 5′ regions contribute to a difference in RNA stability, any difference in transcript levels is due to a relative variation in transcription rate from each start site. The two transcripts were rather closely spaced on the Northern blots. Nevertheless, we were able to detect several instances where a dosage effect upon the 2.3-kb transcript was significantly different from the effect upon the 2.2-kb transcript. The effect of the 1La dosage series in embryo tissue is illustrated in Figure 5. The

ENDOSPERM

B-A	Dose	*cox1*	SE	*cox2*	SE	*cox3*	SE	*atpA*	SE	*rrn18*	SE	*cox5b*	SE
1Sb	hypo	1.01	0.02	1.21	0.06	1.01	0.02	1.02	0.02	1.01	0.04	0.92	0.02
	eup	1.00	0.03	1.00	0.05	1.00	0.01	1.00	0.03	1.00	0.02	1.00	0.06
	hyper	1.45	0.05	1.05	0.02	1.22	0.05	1.17	0.04	1.36	0.09	1.06	0.08
1La	hypo	1.52	0.06	1.09	0.04	0.79	0.01	1.42	0.06	1.15	0.02	1.05	0.01
	eup	1.00	0.01	1.00	0.09	1.00	0.03	1.00	0.02	1.00	0.00	1.00	0.04
	hyper	0.78	0.04	0.96	0.07	1.10	0.03	0.82	0.04	1.02	0.01	1.05	0.04
2Sa	hypo	1.08	0.02	1.01	0.03	1.19	0.03	1.25	0.03	1.00	0.02	1.02	0.01
	eup	1.00	0.01	1.00	0.03	1.00	0.02	1.00	0.02	1.00	0.00	1.00	0.01
	hyper	0.89	0.01	0.82	0.06	0.89	0.02	0.85	0.00	0.90	0.03	1.06	0.02
3La	hypo	1.21	0.09	1.29	0.10	0.92	0.05	1.10	0.11	1.11	0.04	1.04	0.04
	eup	1.00	0.05	1.00	0.15	1.00	0.06	1.00	0.08	1.00	0.03	1.00	0.02
	hyper	1.22	0.14	1.74	0.19	1.08	0.07	1.16	0.12	1.13	0.06	1.03	0.02
4Sa	hypo	1.25	0.04	1.07	0.09	1.14	0.04	1.24	0.04	1.04	0.02	1.03	0.06
	eup	1.00	0.01	1.00	0.06	1.00	0.02	1.00	0.01	1.00	0.03	1.00	0.03
	hyper	1.14	0.03	0.98	0.06	0.99	0.04	0.95	0.12	1.03	0.04	1.04	0.06
4Lb	hypo	0.73	0.02	0.94	0.08	0.74	0.01	0.78	0.02	1.04	0.01	0.95	0.08
	eup	1.00	0.03	1.00	0.02	1.00	0.03	1.00	0.02	1.00	0.02	1.00	0.04
	hyper	0.96	0.08	1.24	0.03	0.88	0.05	0.96	0.02	1.09	0.02	0.93	0.02
5Sc	hypo	1.20	0.04	1.28	0.04	1.10	0.02	1.04	0.01	1.02	0.02	1.01	0.07
	eup	1.00	0.00	1.00	0.04	1.00	0.06	1.00	0.03	1.00	0.02	1.00	0.09
	hyper	0.97	0.02	1.02	0.04	0.92	0.02	1.03	0.02	1.02	0.02	0.97	0.02
5Lb	hypo	1.04	0.06	1.03	0.01	1.04	0.09	1.06	0.03	0.99	0.10	0.76	0.05
	eup	1.00	0.05	1.00	0.02	1.00	0.05	1.00	0.10	1.00	0.26	1.00	0.03
	hyper	1.02	0.08	1.13	0.02	0.81	0.03	0.89	0.09	1.47	0.36	0.95	0.03
6Lc	hypo	1.26	0.01	1.48	0.03	1.35	0.04	1.61	0.04	1.11	0.02	0.82	0.01
	eup	1.00	0.02	1.00	0.04	1.00	0.02	1.00	0.01	1.00	0.06	1.00	0.04
	hyper	0.93	0.02	1.00	0.02	0.96	0.03	0.91	0.03	1.04	0.01	0.95	0.02
7Lb	hypo	1.02	0.06	0.89	0.04	1.01	0.04	0.93	0.02	0.85	0.01	1.03	0.08
	eup	1.00	0.03	1.00	0.03	1.00	0.04	1.00	0.03	1.00	0.02	1.00	0.04
	hyper	1.23	0.07	1.13	0.06	1.19	0.06	1.23	0.04	1.06	0.06	1.09	0.10
8Lc	hypo	1.03	0.05	0.97	0.05	1.10	0.08	0.95	0.02	1.02	0.06	1.06	0.03
	eup	1.00	0.03	1.00	0.08	1.00	0.06	1.00	0.03	1.00	0.07	1.00	0.03
	hyper	1.07	0.05	1.02	0.02	0.96	0.05	0.99	0.02	0.99	0.04	1.03	0.01
9Sd	hypo	1.20	0.04	1.05	0.08	1.20	0.08	1.15	0.05	0.97	0.04	1.04	0.04
	eup	1.00	0.01	1.00	0.08	1.00	0.07	1.00	0.02	1.00	0.03	1.00	0.12
	hyper	1.01	0.03	1.04	0.02	1.02	0.07	0.97	0.02	1.07	0.04	1.25	0.17
9Lc	hypo	1.18	0.05	1.31	0.01	1.11	0.04	1.00	0.02	1.00	0.03	0.86	0.01
	eup	1.00	0.03	1.00	0.03	1.00	0.05	1.00	0.04	1.00	0.01	1.00	0.03
	hyper	1.21	0.08	1.00	0.01	1.06	0.05	1.00	0.04	0.98	0.01	1.02	0.02
10L19	hypo	0.98	0.04	0.96	0.05	1.09	0.04	0.83	0.05	0.82	0.06	0.82	0.04
	eup	1.00	0.01	1.00	0.06	1.00	0.07	1.00	0.05	1.00	0.07	1.00	0.04
	hyper	0.81	0.03	1.10	0.11	0.82	0.07	0.87	0.00	1.00	0.05	0.88	0.06

Figure 3.—The mRNA levels of six genes for 14 dosage series in endosperm tissue. The data are presented in the same manner as in Figure 2.

2.3-kb band appeared to be subject to a stronger negative dosage effect than the 2.2-kb band. The black bars represent the 2.3-kb bands and the gray bars represent the 2.2-kb bands. The 2.3-kb expression levels are shown relative to the 2.3-kb euploid and the 2.2-kb expression levels are shown relative to the 2.2-kb euploid. The 1La dosage series yielded a similar result in the endosperm (Figure 6).

Thirteen aneuploids had a 2.3-/2.2-kb signal ratio that was significantly different from the euploid. Dosage data for these are presented in Figure 6. Shading indicates that the expression level of the aneuploid was significantly different ($P \leq 0.05$, *t*-test) from the euploid. Asterisks indicate that the 2.3-/2.2-kb signal ratio of an aneuploid was significantly different ($P \leq 0.05$, *t*-test) from the euploid 2.3-/2.2-kb signal ratio. In seven cases, the effect upon the 2.2-kb band was less or none (embryo 1La hypoploid and hyperploid, 3La hypoploid, 10L19 hypoploid; endosperm 1La hypoploid, 4Lb hypoploid, and 5Sc hypoploid). For five aneuploids, the effect upon the 2.2-kb band was greater (embryo 4Lb hypoploid, 5Sc hyperploid, 6Lc hypoploid; endosperm 1Sb hypoploid, and 4Sa hypoploid). The 5Sc hypoploid in the embryo was interesting because it resulted in a slight negative effect upon the 2.3-kb transcript and a slight positive effect upon the 2.2-kb band. The actual effects were likely somewhat stronger since the signals of the two closely spaced transcripts partially overlapped, reducing the differences.

Multiple transcripts were also produced by *cox2* and

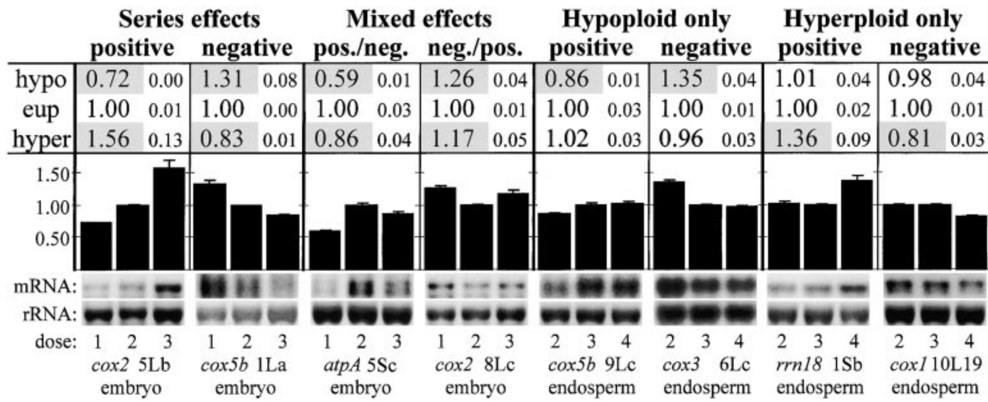

Figure 4.—Examples of chromosomal effects. Each dosage series was composed of hypoploid (hypo), euploid (eup), and hyperploid (hyper) samples. Shaded boxes indicate significant differences from the euploid. Below the numerical values for each example are bar graph representations of the data. Below that the mRNAs and nuclear 26s rRNAs (loading control) from the Northern blots are shown. The gene, dosage series, and tissue of each example are listed at the bottom.

usually *atpA*; in each case only one band was selected for this survey of chromosomal dosage effects (Figures 2 and 3). As a rule, the additional transcripts responded to a dosage series in the same way as the selected transcript. There were several instances where it was apparent that one or more of the additional transcripts responded differently. Unlike *cox1*, no attempt was made to measure the other transcripts for *cox2* and *atpA* because the exact nature of each band is unknown. It is likely that some of the differences in effects observed among the *cox2* transcripts were due to differences in transcription because this gene has at least five separate promoters (Lupold *et al.* 1999a). This could be particularly interesting because transcriptional control of *cox2* appears to involve recombination of the mitochondrial genome, positioning various promoters upstream of the gene (Lupold *et al.* 1999b).

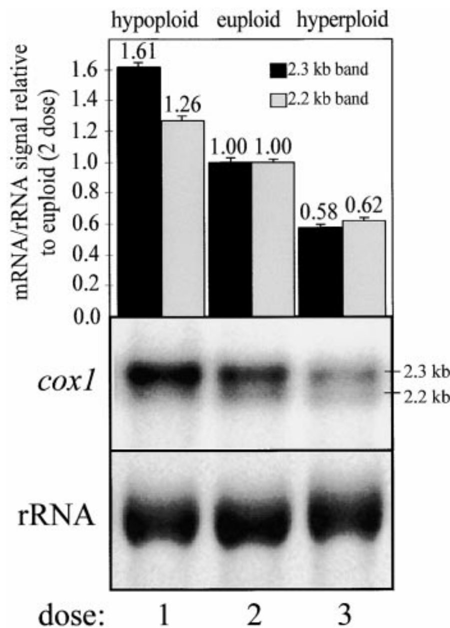

Figure 5.—Effect of 1La dosage series upon the 2.3- and 2.2-kb *cox1* transcripts. The expression levels for the 2.3-kb transcripts (relative to the 2.3-kb euploid level) are shown as solid bars. The expression levels for the 2.2-kb transcripts (relative to the 2.2-kb euploid level) are shown as shaded bars. The mRNAs and rRNAs for each dosage are shown below.

Dosage series effects upon *cox5b*: Effects upon *cox5b* (Figures 2 and 3) were included for comparison. This component of the cytochrome c oxidase complex is encoded in the nucleus. Because the chromosomal location of *cox5b* is unknown in maize, it is possible that its copy number may have been varied in one of the dosage series. Transcript levels of *cox5b* were affected by a number of dosage series, but none of them displayed a structural gene dosage effect. A structural gene dosage effect would have been indicated if expression levels correlated to gene dosage; *e.g.*, one dose resulted in an expression level that was 50% of two doses. Failure to detect a structural gene dosage effect does not eliminate the possibility that *cox5b* is located on one of the B-A's used in this study. It appears that in maize many genes are subject to dosage compensation in whole chromosome arm aneuploidies (Guo and Birchler 1994). Dosage compensation occurs when a dosage-dependent negative modifier is varied concurrently with the structural gene (Birchler 1981; Birchler and Newton 1981).

In general, the extent and type of effects seen for *cox5b* appeared to be similar to the mitochondrial genes with one notable difference. Whereas mitochondrial genes mostly experienced negative effects in both embryo and endosperm, the effects upon *cox5b* in the two tissues were different. Embryo effects were mostly negative (five positive *vs.* eight negative) while endosperm effects were positive (five positive *vs.* zero negative). As with the mitochondrial genes, effects were generally tissue specific. Only 10L19 showed similar effects in both the embryo and the endosperm.

Dosage effects of TB-10L32 *vs.* TB-10L19: The dosage series of TB-10L19 involved aneuploidy of the whole long arm of chromosome 10 (10L). For comparison we obtained data from a TB-10L32 dosage series, where only the distal 26% of 10L was varied in copy number (Maguire 1985). Whereas 10L19 affected four genes in the embryo and three in the endosperm, 10L32 affected

EMBRYO

B-A	Dose	2.3 kb	SE	2.2 kb	SE
1La	hypo	1.61*	0.04	1.26*	0.04
	eup	1.00	0.03	1.00	0.03
	hyper	0.58*	0.01	0.62*	0.01
3La	hypo	1.15*	0.08	1.07*	0.08
	eup	1.00	0.06	1.00	0.04
	hyper	1.12	0.05	1.10	0.05
4Lb	hypo	1.07*	0.04	1.19*	0.01
	eup	1.00	0.01	1.00	0.02
	hyper	1.12	0.03	1.11	0.02
5Sc	hypo	1.06*	0.02	0.94*	0.01
	eup	1.00	0.00	1.00	0.00
	hyper	1.01*	0.08	1.06*	0.08
6Lc	hypo	1.00*	0.05	1.22*	0.07
	eup	1.00	0.02	1.00	0.02
	hyper	1.09	0.05	1.06	0.05
10L19	hypo	1.21*	0.07	1.09*	0.04
	eup	1.00	0.04	1.00	0.03
	hyper	1.04	0.04	0.96	0.05

ENDOSPERM

B-A	Dose	2.3 kb	SE	2.2 kb	SE
1Sb	hypo	1.01*	0.02	0.94*	0.01
	eup	1.00	0.03	1.00	0.03
	hyper	1.45	0.05	1.35	0.02
1La	hypo	1.52*	0.06	1.13*	0.07
	eup	1.00	0.01	1.00	0.02
	hyper	0.78	0.04	0.82	0.05
4Sa	hypo	1.25*	0.04	1.37*	0.08
	eup	1.00	0.01	1.00	0.03
	hyper	1.14	0.03	1.04	0.05
4Lb	hypo	0.73*	0.02	0.81*	0.02
	eup	1.00	0.03	1.00	0.07
	hyper	0.96	0.08	1.02	0.04
5Sc	hypo	1.20*	0.04	1.10*	0.03
	eup	1.00	0.00	1.00	0.02
	hyper	0.97	0.02	0.97	0.03

Figure 6.—Comparison of 2.3- and 2.2-kb *cox1* transcripts. Data are shown for those dosage series where the 2.3-/2.2-kb transcript signal ratio of an aneuploid was significantly different ($P \leq 0.05$, *t*-test) from the euploid (indicated by asterisks). Shaded boxes indicate aneuploid expression levels that are significantly different from the euploid ($P \leq 0.05$, *t*-test).

only one gene (*cox5b*) and this was in both tissues (Figure 7). These results indicate that the factor or factors involved with the dosage effects on *cox1*, *cox3*, and *atpA* are on the proximal 74% of 10L. The dosage effects on *cox5b* may be explained by a factor on the distal 26% of 10L.

DISCUSSION

This study establishes that multiple regions of the nuclear genome affect mitochondrial gene expression levels. A change in transcript abundance in a dosage series indicates the presence of one or more *trans*-acting factors on the affecting chromosome arm. Guo and Birchler (1994) conducted a similar study of B-A dosage effects upon six nuclearly encoded genes. In that study each gene was affected by an average of six dosage series. This number is close to the average of seven for mitochondrial genes, especially considering that there was one less dosage series (9Lc) in the nuclear gene study. As with the mitochondrial genes, most of the effects upon nuclear genes were negative (37 positive *vs.* 53 negative). However, when embryo and endosperm effects were considered separately, the profile of effects differed. While embryo effects were predominantly negative (5 positive *vs.* 33 negative), endosperm effects were mostly positive (32 positive *vs.* 20 negative). The profile of effects upon the nuclearly coded *cox5b* appears to follow the trend of the other nuclear genes (Table 3). At least in the endosperm, the effects indicate that mitochondrial genes are subject to a different mode of dosage-sensitive effects than are nuclear genes, which may indicate a different mode of regulation.

The results demonstrate that there are numerous dosage-dependent nuclear factors that affect mitochondrial transcript levels; these modulations might result from a variety of mechanisms. Transcript abundance is affected not only by transcription and turnover rates, but also potentially by a change in mitochondrial genome copy number. This could be due to variation in the number of mitochondria per cell or a change in the copy number of genomes per mitochondrion. In the formation of the maize tapetum, cells can experience up to a 40-fold

B-A	Dose	EMBRYO *cox5b*	SE	ENDOSPERM *cox5b*	SE
10L19	hypo	0.75	0.04	0.82	0.04
	eup	1.00	0.06	1.00	0.04
	hyper	1.00	0.06	0.88	0.06
10L32	hypo	0.73	0.04	0.70	0.01
	eup	1.00	0.03	1.00	0.03
	hyper	1.07	0.02	0.85	0.07

Figure 7.—Effects of 10L19 and 10L32 upon *cox5b* in the embryo and endosperm. The data are presented in a similar manner as in Figure 2. The 10L19 dosage series involves all of 10L. The 10L32 dosage series involves only the distal 26% of 10L.

TABLE 3

Summary of pairwise effects

Genes	Embryo Positive	Embryo Negative	Endosperm Positive	Endosperm Negative
Mitochondrial	14	28	21	29
cox5b	5	8	5	0
Nuclear	5	33	32	20

increase in the number of mitochondria per cell (Lee and Warmke 1979). Differences in mitochondrial numbers have been described in other maize tissues (Clowes and Juniper 1964; Juniper and Clowes 1965). However, such copy number differences might be expected to result in a global change among all of the mitochondrial gene transcript levels. No strong global effects were apparent.

This result does not rule out the possibility that a dosage-sensitive nuclear factor could alter mitochondrial gene copy number. A global effect due to a change in mitochondrial genome number could be masked if a second factor on a chromosome arm had a specific effect upon one of the mitochondrial genes being assayed. Alternatively, changes in gene copy number may not have involved the whole mitochondrial genome but, instead, some portion of it. The organization of the maize mitochondrial genome is complex; subgenomes are formed through intramolecular recombination (Fauron *et al.* 1995). Differential amplification of a subgenome would change the copy number of only a subset of genes.

Mitochondrial ribosomal RNA (*rrn18*) was included in the study with the expectation that it would be relatively insensitive to modulation. Indeed, of all the genes assayed, *rrn18* had the least number of effects and most of those were modest. If a particular aneuploidy affected a number of the mitochondrial genes including *rrn18*, it could have been an indication that mitochondrial genome copy number was being affected. Hyperploid 1Sb in endosperm had the same effect upon the RNA levels of all of the mitochondrial genes except *cox2*. Also in the endosperm, hyperploid 2Sa had negative effects upon all of the tested mitochondrial genes while hypoploid 2Sa had negative effects upon *cox1*, *cox3*, and *atpA*. However, the effects of 1Sb or 2Sa were not particularly strong. If any dosage series affected mitochondrial genome copy number, it did not appear to contribute greatly to the effects upon RNA levels.

The 6Lc dosage series was interesting in that it may have indicated a different trend. Although it had no effect upon *rrn18*, the 6Lc hypoploid negatively affected all of the other tested mitochondrial genes. While the 2.3-kb *cox1* transcript was not affected in the embryo, the 2.2-kb transcript was. In addition, the 6Lc hyperploid had a negative effect upon *cox3* and *atpA* in the embryo and *cox1* in the endosperm. It would be interesting to determine if these effects were the coincidental result of multiple factors or were due to a single factor with a general effect upon mitochondrial mRNA levels.

A related question concerns the possibility of coordinate regulation of genes that encode functionally related proteins. In yeast the cytochrome c oxidase complex utilizes the various subunits in an equimolar stoichiometric ratio (Capaldi 1990; Droste *et al.* 1996). Transcriptional regulation of these genes is coordinated at least in part by the modulation of the level of the nuclearly encoded mitochondrial RNA polymerase (Ulery *et al.* 1994). Our data offered little evidence that dosage-sensitive factors were involved in the coordinate regulation of the RNA levels in maize. The functionally related *cox1, cox2,* and *cox3* genes were no more likely to have coincidental effects with each other than they were with *atpA*. A possible exception was the effect of the 5Sc hypoploid. It had a negative effect upon all three *cox* genes in the embryo. Coincidentally, it also had a negative effect upon *cox1* and *cox2* in the endosperm. The other mitochondrial genes did not share these effects, nor did nuclearly encoded *cox5b*.

A well-characterized dosage phenomenon relating to the kernel concerns endosperm size. Six of the B-A chromosomes used to create the dosage series carry endosperm size factors (*Ef*; Birchler 1993). Endosperms that are hypoploid for 1Sb, 1La, 4Sa, 5Sc, 7Lb, and 10L19 are markedly smaller than euploid endosperm. The data were examined as to whether effects upon mitochondrial genes could be responsible for the reduced size of hypoploid endosperms. The effects detected in this study did not correlate with *Ef*s. Although this does not rule out the possibility that one or more *Ef*s may result in reduced kernel size because of a limitation of mitochondrial function or a general reduction in mitochondrial gene expression, it seems unlikely that *Ef*s are primarily due to a modulation of mitochondrial transcript level.

Nevertheless, it is possible that a limitation of mitochondrial function contributes to aneuploid syndromes. Indeed, in plants heteroplasmic for mutant mitochondria, the proportion of functional mitochondria correlates with general plant vigor (Marienfeld and Newton 1994). Because there are decreases of gene expression in both hypoploids *and* hyperploids, it is conceivable that these reductions become rate limiting on growth. This can explain the observation that aneuploids have less vigor than do their euploid siblings (Birchler and Newton 1981; Guo and Birchler 1994).

The multitude of effects most likely reflects a hierarchy of dosage-dependent processes. The molecular identification of individual genes that are responsible for such dosage effects indicates a heterogeneous collection of regulatory genes as the responsible agents (*e.g.*, Henikoff 1996; Frolov *et al.* 1998). It appears, therefore, that many regulatory mechanisms have evolved to operate in such a manner and at such a level as to be rate limiting on the phenotype. Genes at the top of the hierarchy will still affect the phenotype because the dosage effect will be transmitted through a series of dosage-dependent steps to the ultimate monitored phenotype. This concept unifies the polygenic nature of additive quantitative traits and the extensive effects of aneuploidy on any one characteristic. The large number of modulations of mitochondrial gene expression found in this study is consistent with this view. Also, this type of hierarchy explains a similar spectrum of effects on

mitochondrial and nuclear genes despite a fundamentally different chromatin configuration; the hierarchy potentially operates through a series of steps involving nuclear genes before the ultimate impact on mitochondrial expression.

In summary, numerous chromosomal dosage effects were found that resulted in the modulation of the RNA levels of mitochondrial genes. The array of dosage effects upon mitochondrial genes was similar to what had been previously found with nuclear genes. For the most part modulations were tissue specific. A dosage series that affected transcript levels of a gene in embryo tissue usually had a different effect in the endosperm. Most effects were negative; *i.e.*, increased chromosome copy number resulted in lower gene expression levels. This similarity in the array of effects indicates that although mitochondrial genes are prokaryotic in origin, their regulation has been extensively integrated into the eukaryotic cell. Even so, the modes of regulation for mitochondrial and nuclear genes are not identical. A notable difference was that nuclear genes experienced mostly positive effects in the endosperm while the effects upon mitochondrial genes were mostly negative. There is little evidence of coordinate regulation of the *cox* genes at the RNA level.

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