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Influence of homoeologous chromosomes on gene-dosage effects in allohexaploid wheat (*Triticum aestivum* L.)

(polyploid evolution/gene expression/endosperm proteins/electrofocusing–electrophoresis)

C. ARAGONCILLO, M. A. RODRÍGUEZ-LOPERENA, G. SALCEDO, P. CARBONERO, AND F. GARCÍA-OLMEDO*

Departamento de Bioquímica, Escuela Técnica Superior de Ingenieros Agrónomos, Madrid-3, Spain

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ABSTRACT **Gene-dosage responses for a group of six wheat endosperm proteins have been investigated by using compensated nulli-tetrasomic lines of cv. Chinese Spring. Practically linear dosage responses have been observed for all the proteins. However, for two of the proteins (and probably for a third one), the net output of protein, at each dosage of its structural gene, was 30–80% higher when the chromosome carrying an active homoeogene was absent. The possible significance of this effect in connection with the loss of gene redundancy undergone by polyploids is discussed.**

A linear correlation has been assumed to exist between structural gene dosage and the amount (or activity) of the corresponding protein for most eukaryotic systems investigated, in organisms as varied as *Saccharomyces* (1–3), *Datura* (4), barley (5), *Drosophila* (6–8), and different mammals (9–12) including man (13, 14). The allohexaploid wheat *Triticum aestivum* L. (genomes AABBDD) is an excellent material for study of gene-dosage responses because of the availability of the compensated nulli-tetrasomic lines developed by Sears (15, 16) from the cultivar Chinese Spring. In each of these lines, a pair of homoeologous chromosomes from one genome is substituted for by the corresponding pair from one of the other two homoeologous genomes (ancestral homologues). For all systems controlled by triplicate genes, located in homoeologous chromosomes, the overall gene-dosage is the same in the compensated nulli-tetrasomic lines as in the euploid. On the other hand, for those systems controlled by duplicate genes (third locus absent or silent), the different nulli-tetrasomic lines have different overall dosages, depending on whether there are 0, 2, or 4 doses (0, 3, or 6 doses in endosperm, which is triploid) of the chromosome that do not express information for the system. If the gene products corresponding to each locus are distinguishable, electrophoretically or otherwise, the output of each gene can be studied as a function of its own dosage and of the dosages of its homoeologues. Although dosage effects have been repeatedly observed in connection with the investigation of chromosome–protein associations in wheat (17–20), they have not been specifically investigated in a quantitative way.

Systems that do not fit a linear gene-dosage response have been also reported, most notably the dosage compensation that affects genes located in the sex chromosomes of organisms such as *Drosophila* (see ref. 21) and the competitive expression of allelic variants of alcohol dehydrogenase in maize (22). Deviations from linearity have been suspected in a few of the cases investigated in wheat (23–26), but no quantitative evidence has been presented.

We report here a quantitative study of gene-dosage response in a group of previously described endosperm proteins (27, 28) which are controlled by incomplete (not triplicate) homoeologous gene sets located in homoeologous chromosome groups 3, 4, and 7.

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MATERIALS AND METHODS

Wheat Stocks. Compensated nulli-tetrasomic lines from the *Triticum aestivum* cv. Chinese Spring, involving chromosomes from groups 3, 4, and 7—with the exception of those nullisomic for 4A (sterile or nearly so)—were the gift of E. R. Sears (Columbia, MO), who also provided ditelosomic 4Aα (15, 16).

Protein Extraction and Fractionation by Combined Electrofocusing/Electrophoresis. Kernels were crushed between two polished metal plates with the aid of a hammer, and lipid was extracted with about 25 vol of petroleum ether (bp 35–60°) for 2 hr. Most of the solvent was eliminated with a syringe, and the rest was allowed to evaporate from the residue at room temperature. The proteins were extracted three times with 70% ethanol (10 + 10 + 10, vol/wt) and the solvent of the combined extracts was evaporated under reduced pressure at room temperature. The proteins were redissolved in 25 volumes of 9 M urea. An appropriate aliquot of the extract was incorporated into the electrofocusing polymerization mixture.

Combined electrofocusing/electrophoresis was carried out by a method (27) based on that of Wrigley (29). Electrofocusing (pH range 5–8) was performed in 2 × 140 mm polyacrylamide gel columns; electrophoresis (pH 3.2), in the second dimension was carried out in thin (2 mm) starch gels. Staining of proteins was performed with water-soluble 0.05% Nigrosine (Fluka catalog no. 72470) in methanol/water/acetic acid, 5:5:1 (vol/vol) for 14–16 hr; destaining was with 70% ethanol after rinsing with tap water.

Quantitation of Components from the Protein Map. This was done by reflectance densitometry using a Chromoscan densitometer (Joyce Loebl) with a 654-nm filter. Preliminary experiments were carried out to establish optimal conditions for quantitation. Peak height was found to be much more reproducible ($S/\overline{X} < 0.05$ for triplicates) than peak area ($S/\overline{X} > 0.10$), due to proximity of some spots. Peak height of the proteins identified in Fig. 1, except protein 5, varied linearly with the amount of protein for extract loads representing up to 50 mg of euploid ground kernel. Component 5, which was of interest for our study, could not be quantitated due to improper staining with Nigrosine (unstained area in the center of the spot). The following procedure was finally adopted. Four genetic stocks were compared in a typical experiment. Quadruplicate samples of each stock were subjected to combined electrofocusing/electrophoresis in parallel and stained with the same lot of Nigrosine solution. Sample size was that corresponding to 20 mg of ground kernel, to allow more than dou-

* To whom reprint requests should be addressed.

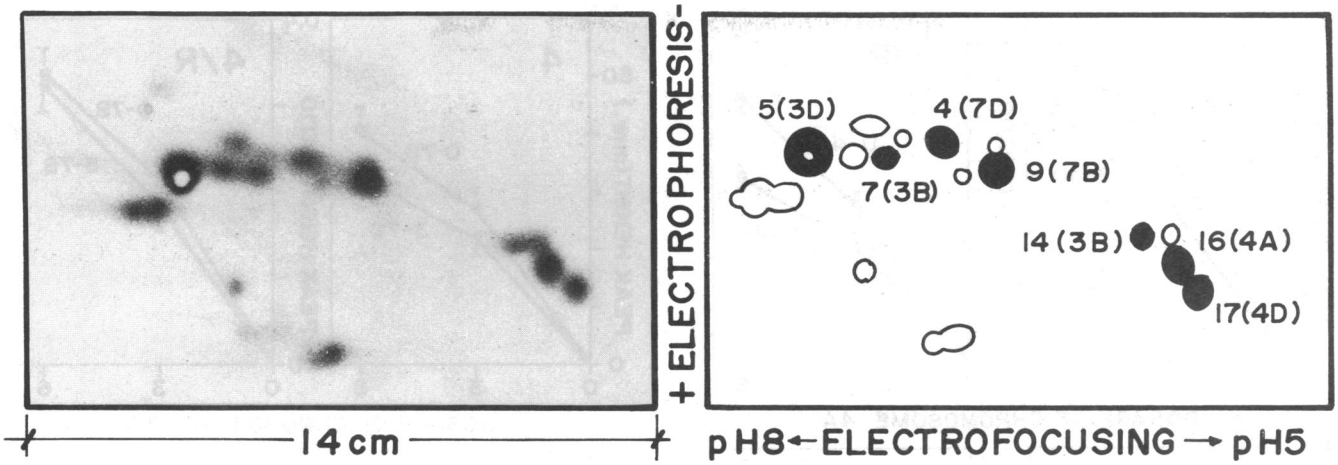

FIG. 1. (*Left*) Two-dimensional map of proteins extracted from wheat endosperm by 70% ethanol and fractionated by combined electrofocusing/electrophoresis (27, 28). (*Right*) Diagram of the map: black spots correspond to the proteins selected for the present study, which are identified by numbers; the chromosomes controlling each of these proteins are indicated in parenthesis.

bling of a specific protein, with respect to the euploid level, without going out of the linearity range.

RESULTS

Chromosomal Control and Characterization of the Proteins under Study. The chromosomal control of the proteins under study (Fig. 1) has been established and their characterization has been partially achieved (27, 28). A summary of their most salient characteris is pertinent here.

The purification and characterization of components 16 and 17 of the two-dimensional map, which are encoded by genes located in chromosomes 4A and 4D, respectively, will be reported elsewhere. The evidence indicating their close relationship (homoeology) is presented in Table 1.

Table 1. Amino acid composition and molecular weight of components 16 and 17*

Amino acid	Protein 16†	Protein 17†
Lysine	2	2
Histidine	2	1
Arginine	6	5
Aspartic acid	7	7
Threonine	6	6
Serine	6	6
Glutamic acid	21	21
Proline	12	14
Glycine	6	7
Alanine	3	4
Valine	3	3
Methionine	2	3
Isoleucine	4	4
Leucine	10	9
Tyrosine	3	3
Phenylalanine	2	2
Total no. of residues	95	97
Minimum mol wt†	10,751	10,798
$NaDodSO_4$ mol wt‡	11,800	11,850

* From G. Salcedo, M. A. Rodríguez-Loperena, and C. Aragoncillo, unpublished results.

† The minimum molecular weight and the corresponding number of residues of each amino acid have been calculated from analytical data by the computer method of Delaage (30). Half-cystine and tryptophan were not analyzed and, therefore, are not included in the calculation.

‡ Molecular weight determined by sodium dodecyl sulfate electrophoresis as described in ref. 31.

The homoeology of proteins 4 and 9, encoded by genes located in chromosomes 7D and 7B, respectively, has been established on the basis of their similar amino acid composition (23, 32), peptide map (19, 32), molecular weight (23, 32), and solubility in chloroform/methanol, 2:1 (vol/vol), and in 70% ethanol (23, 27, 32).

The relationship between protein 5, encoded by a gene located in chromosome 3D, and proteins 7 and 14, encoded by genes located in chromosome 3B, is not well established. These proteins are soluble in 70% ethanol and in water but not in chloroform/methanol, and they could be identical with some amylase inhibitors (33). Apart from this, it is unknown whether component 5 is homoeologous with component 7, with component 14, or with neither.

No traces of any of the proteins included in this study were detected in the ground kernel residue after three extractions with 70% ethanol, so it was concluded that the extraction by this solvent was quantitative.

Gene-Dosage Effects. Gene-dosage (or chromosome-dosage) effects on proteins 16 and 17 are shown in Fig. 2. The amount of protein 16, for a given dosage of the chromosome carrying the structural gene (4A), is not constant, whether it is expressed relative to dry matter or relative to the amount of proteins 4 and 9, encoded by genes in chromosomes 7D and 7B, whose dosages are constant in the compared stocks. At 3 doses of chromosome 4A, the ranking of the stocks with respect to protein 16 is nulli-4D tetra-4B > euploid = nulli-4B tetra-4D; at 6 doses, the ranking is nulli-4D tetra-4A > nulli-4B tetra-4A. At both dosages, the stocks with the higher level of protein 16 are those lacking chromosome 4D, which carries the structural gene for the homoeologous protein 17. As shown in Fig. 2, quasi-linear relationships exist between the protein level and the structural gene dosage for stocks lacking chromosome 4D and for those carrying it. Because the compensated nulli-tetrasomics involving chromosome 4A as nullisomic are not available, it has not been possible to ascertain if the same phenomenon is affecting protein 17. However, the result obtained for ditelosomic 4Aα, which lacks the structural gene for protein 16, seems to indicate that this might be the case, because the ranking of stocks with 3 doses of chromosome 4D, which carries the structural gene for protein 17, with respect to the amount of this protein (relative to proteins 4 and 9) is ditelo-4Aα > euploid = nulli-4B tetra-4A. The amounts, on a dry matter basis, of all proteins in the map of Fig. 1 was lower in ditelo-4Aα than in the euploid.

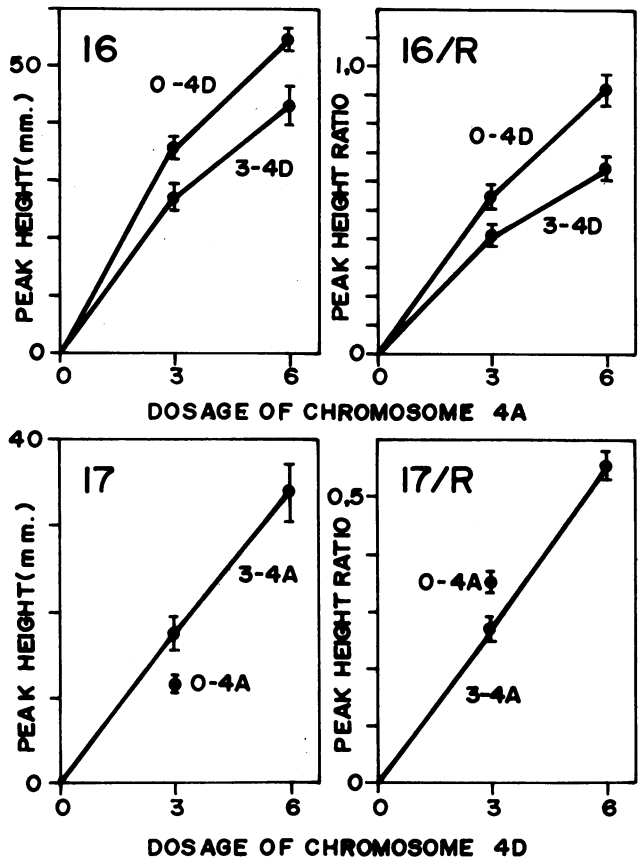

FIG. 2. Chromosome-dosage responses for proteins 16 and 17, encoded by genes located in chromosomes 4A and 4D, respectively. Protein 16: ditelo-4Aα (0 doses of structural gene), euploid (3-4A, 3-4B, 3-4D, in triploid endosperm), nulli-4D tetra-4B (3-4A, 6-4B, 0.-4D), nulli-4B tetra-4A (6-4A, 0-4B, 3-4D), nulli-4D tetra-4A (6-4A, 3-4B, 0-4D). Values for protein 16 in nulli-4B tetra-4D (3-4A, 0-4B, 6-4D) and in the euploid (3-4A, 3-4B, 3-4D) did not differ significantly (measured in experiment for protein 17; not represented in the graphs). Protein 17: nulli-4D tetra-4A and nulli-4D tetra-4B (0 doses of structural gene), euploid (3-4A, 3-4B, 3-4D), ditelo-4Aα (0-4Aβ, 3-4B, 3-4D), nulli-4B tetra-4D (3-4A, 0-4B, 6-4D). Values for protein 17 in nulli-4B tetra-4A (6-4A, 0-3B, 3-4D) and in the euploid (3-4A, 3-4B, 3-4D) did not differ significantly (measured in experiment for protein 16; not represented in the graphs). (*Left*) Densitometric peak height corresponding to 20 mg of original sample of each of the stocks. (*Right*) The same values divided by R (sum of peak heights of proteins 4 and 9). Values are means ± SEM of four determinations. Dosage responses and differences at each dosage are all statistically significant ($P < 0.05$).

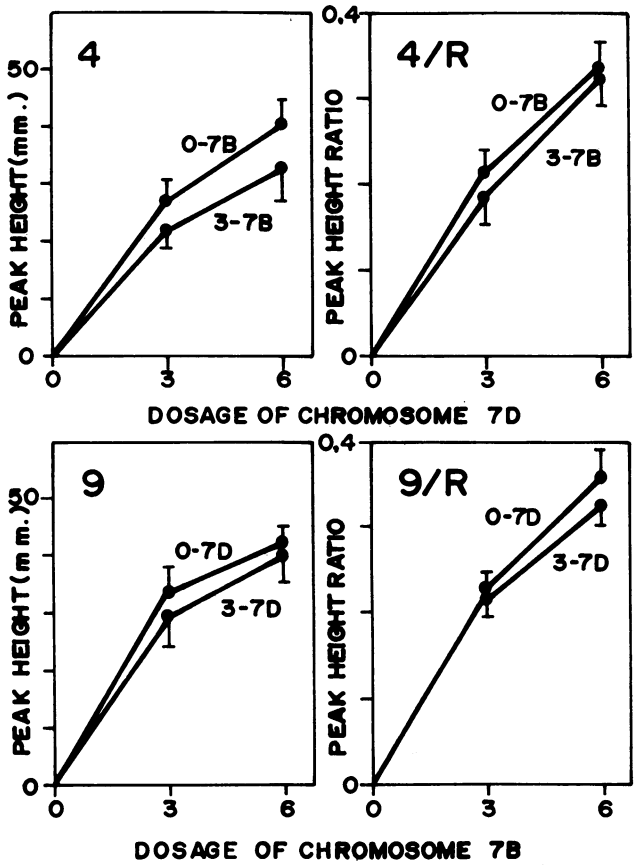

FIG. 3. Chromosome-dosage responses for proteins 4 and 9, encoded by genes located in chromosomes 7D and 7B, respectively. Protein 4: nulli-7D tetra-7A and nulli-7D tetra-7B (0 doses of structural gene), euploid (3-7A, 3-7B, 3-7D), nulli-7B tetra-7A (6-7A, 0-7B, 3-7D), nulli-7A tetra-7D (0-7A, 3-7B, 6-7D), nulli-7B tetra-7D (3-7A, 0-7B, 6-7D). Values for protein 4 in nulli-7A tetra-7B (0-7A, 6-7B, 3-7D) and in the euploid (3-7A, 3-7B, 3-7D) did not differ significantly (measured in experiment for protein 9; not represented in the graphs). Protein 9: nulli-7B tetra-7A and nulli-7B tetra-7D (0 doses of structural gene), euploid (3-7A, 3-7B, 3-7D), nulli-7D tetra-7A (6-7A, 3-7B, 0-7D), nulli-7A tetra-7B (0-7A, 6-7B, 3-7D), nulli-7D tetra-7B (3-7A, 6-7B, 0-7D). Values for protein 9 in nulli-7A tetra-7D and in euploid (3-7A, 3-7B, 3-7D) did not differ significantly (measured in experiment for protein 4; not represented in the graphs). R = sum of peak heights of proteins 7, 14, 16, and 17. Dosage responses were significant ($P < 0.05$) and differences between stocks at each dosage were not significant. Other details as in Fig. 2.

Gene dosage-response data for the homoeologous proteins 4 and 9 are presented in Fig. 3. In both cases, no significant differences were found in the amount of protein, at a given dosage of the structural gene, among the different stocks. Again, a quasi-linear gene-dosage response was observed.

In Fig. 4, the gene-dosage responses of proteins 7 and 14, whose structural genes are located in chromosome 3B, are represented. The case of protein 14 seems to be similar to that of protein 16, and that of protein 7 is identical to the cases of proteins 4 and 9.

DISCUSSION

Gene-Dosage Responses. The linear gene-dosage responses that have been generally found for most eukaryotic systems (1–14, 17–20) have been interpreted as meaning that transcription is generally the rate-limiting step in eukaryotic gene expression, the rate of transcription being constant for each structural gene in a given differentiated tissue and independent of the number of copies of the gene (4). Although a quasi-linear gene-dosage response has been found in all cases included in the present report, in some of them the amount of gene product for a given dosage is not the same in different genetic stocks (Figs. 2 and 4). In these cases, the stocks with the higher level of a protein, at each dosage of its structural gene, are those lacking the chromosome that seems to carry the active homoeologous gene. Because the observations have been carried out in compensating nulli-tetrasomics, concomitant dosage changes of the third homoeologue (the one that apparently does not carry an active homoeologous gene) can not be excluded *a priori* as potentially responsible for the observed increase in the amount of the protein. However, when the results are analyzed in terms of the third homoeologue, no general pattern emerges, so only the possibility of interaction between the

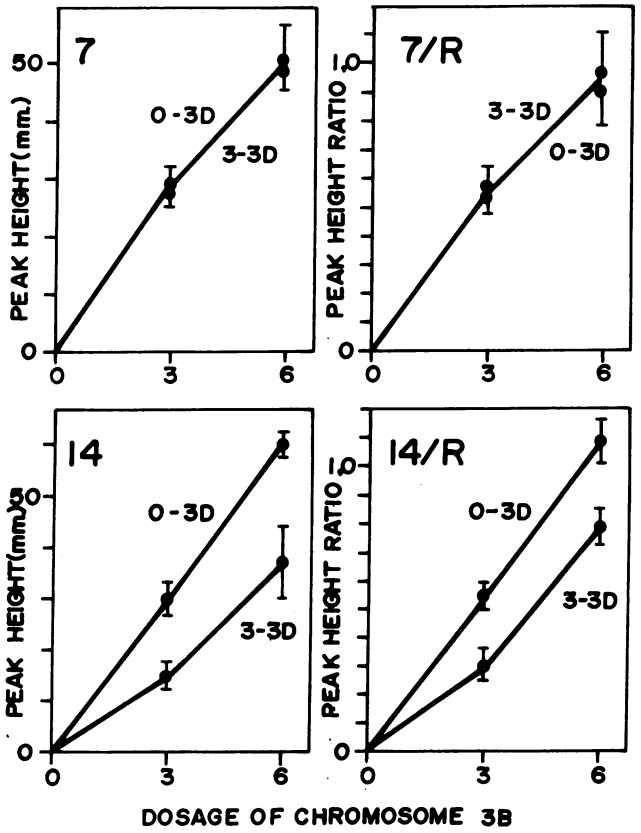

FIG. 4. Chromosome-dosage responses for proteins 7 and 14, encoded by genes located in chromosome 3B (protein 5 could not be measured because of technical reasons): nulli-3B tetra-3A and nulli-3B tetra-3D (0 doses of structural genes), euploid (3-3A, 3-3B, 3-3D), nulli-3D tetra-3A (6-3A, 3-3B, 0-3D), nulli-3A tetra-3B (0-3A, 6-3B, 3-3D), nulli-3D tetra-3B (3-3A, 6-3B, 0-3D). Values for 7 or 14 in nulli-3A tetra-3D (0-3A, 3-3B, 6-3D) and in the euploid (3-3A, 3-3B, 3-3D) did not differ significantly (measured in a separate experiment; not represented in the graphs). R = sum of peak heights of proteins 16 and 17. Dosage responses for both proteins and differences at each dosage for protein were significant ($P < 0.05$). Differences at each dosage for protein 7 were not significant. Other details as in Fig. 2.

homoeologues carrying active genes will be considered in our discussion.

Because we have been actually dealing with changes in chromosome dosage, the possibility that the dosage of regulatory genes might be different in some compensated nulli-tetrasomic lines with respect to the euploid has to be considered. It has been proposed that "superrepression" mechanisms operate in eukaryotes (34, 35) and that, due to this fact, extra chromosomes could result in decreased, unchanged, or increased genetic expression in the case of "regulated" genes depending on the concentrations of specific inducers, whereas "nonregulated" (constitutive) loci would be expected to show increased expression. The fact that, in all cases studied, the expression at the disomic level is not decreased by increasing the dosage of the chromosome carrying the homoeologous gene excluded an explanation of the present results in terms of a superrepression mechanism, such as that proposed by Yielding (34), or in terms of a competitive mechanism of the type postulated for maize alcohol dehydrogenase by Schwartz (22). Whatever mechanism is involved, it does not seem to be affecting the whole chromosome, because one of the systems associated with chromosome 3B shows the phenomenon while the other does not (Fig. 4).

In conclusion, we describe here cases in which the level of a protein is strictly determined by the dosage of its structural gene and cases in which it is not. In the latter cases, it seems that, although there are linear gene-dosage responses, the output of protein per gene dose is higher in the absence of the chromosome carrying the active homoeogene. This situation would have some similarity to the gene-dosage compensation operating in *Drosophila*, where the activity per dose of X-linked genes in the male (XY) is double that in the female (XX) and the activity per cell increases linearly with dosage, both in males and in females (see ref. 21). However, it should be pointed out that the two phenomena differ in some important features. Most notably, in our case, homoeologous chromosomes (rather than homologous) are involved and the increase in activity is only of the order of 30–80%.

Evolutionary Implications. Polyploidization, together with segmental (tandem) duplication, has played an important evolutionary role, as an escape from the constraints to mutation in vital genes, and has occurred extensively in the evolution of early vertebrates (fish and amphibian) and of plants (see ref. 35).

Several lines of evidence indicate that, after the polyploid formation, a diploidization process is started whose main features are: (*i*) the change to a diploid meiotic behavior, achieved by structural rearrangement of chromosomes (see ref. 35) or by the action of diploidizing genes (36–38); (*ii*) the evolution of some redundant genes toward different functions or different developmental specificities (see refs. 35, 39); (*iii*) the loss of redundant genetic activity (see refs. 39–41).

As a consequence of the last two aspects of the process, the effective gene dosage for the systems involved is reduced. Recent estimates of the fraction of the genome affected by this reduction in fish (39) and in plants (our unpublished calculation) indicate that it can be quite important—i.e., as little as 35% of duplicate genes expressed in the most advanced tetraploid Catostomidae fish (39).

The dosage ratios existing in the diploid state between functionally related genes are initially maintained in the polyploid. The increase in cell size, normally associated with the increase in ploidy level, implies a decrease of the surface/volume ratio and, thus, might reduce the relative requirement of certain gene products—e.g., some membrane enzymes (42, 43). Nevertheless, even in these more favorable cases, the loss of duplicate (or triplicate) gene expression has to be considered as potentially disruptive of the balance of dosages for functionally related genes. This deleterious effect would tend to counteract whatever factors favor gene-dosage reduction, unless the amount of gene product has little effect on the overall rate of the process in which it participates or unless some sort of adjustment of the output of protein per gene dose is operating for the system. The present report of such type of adjustment among sets of homoeologous genes that already have undergone one step of dosage reduction, from triplicate to duplicate, would support the above views. However, due to the limited number of sets examined, it would be premature to judge whether this phenomenon occurs generally in connection with diploidization of polyploids.

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