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Nuclear DNA content in F_1 hybrids of maize

A. LANE RAYBURN, D. P. BIRADAR, D. G. BULLOCK AND L. M. MCMURPHY
Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

The nuclear DNA content was determined in two separate experiments for 19 maize inbred lines and 26 maize F_1 hybrids. Ten inbred lines were initially screened in the first experiment. An 11 per cent difference in nuclear DNA amount was observed between the two lines with the lowest amounts of DNA and two lines with the largest genome sizes. All possible hybrid combinations were made among these four lines. In all cases, the genome sizes of the F_1 hybrids were not significantly different from their expected parental means. In several cases, however, F_1 plants of specific crosses were not uniform in genome size. In these crosses, genome sizes observed ranged from the genome size of the low parental genome to the largest parental genome. These results indicate an instability in F_1 genome sizes in certain maize crosses. In order to corroborate this hypothesis, a second experiment was performed. To remove any biases with respect to genome size, 14 F_1 maize hybrids were selected solely on the basis of their heterotic response with no regard for their genome sizes or the genome sizes of their parental inbred lines. The nuclear DNA content of the nine parental lines and 14 hybrids was determined. In most of the crosses, the nuclear DNA content of the F_1 hybrids was not significantly different from their respective parental means. However, in five parental combinations, the mean nuclear DNA content of the F_1 hybrids was significantly higher than their respective parental means. The combined results of this study support the hypothesis of instability in nuclear DNA content in F_1 hybrids of maize. This instability appears not to be universal in all maize hybrids but is restricted to specific parental combinations.

Keywords: flow cytometry, genome size, inheritance, *Zea mays*.

Introduction

Intraspecific genome size variation has been well documented in *Zea mays* ssp. *mays* (Laurie & Bennett, 1985; Rayburn *et al.*, 1985; Rayburn & Auger, 1990; Rayburn, 1990; McMurphy & Rayburn, 1991). Along with establishing the variability of nuclear DNA content in maize, these studies have speculated that nuclear DNA content may be of possible adaptive significance. In maize, genome size has been correlated with various parameters including latitude and/or altitude of adaptation (Rayburn *et al.*, 1985; Rayburn, 1990). Bullock & Rayburn (1991) hypothesized that the relationship between altitude and genome size was really a reflection of a correlation between length of effective growing season at various altitudes and genome size. The basis of this hypothesis is Bennett's proposed model of nucleotypic effects of genome size on plant growth and development (Bennett, 1972). Ho & Rayburn (1991) also observed a positive correlation between chloroplast number and genome size in maize and attributed it to nucleotypic effects of genome size. If nucleotypic factors are important in the adaptation of maize to given environments, how nuclear DNA content is determined from generation to generation needs to be addressed.

Hutchinson *et al.* (1979) crossed several species of *Lolium* that differed by approximately 40 per cent in nuclear DNA amount. They observed that the nuclear DNA contents of the F_1 hybrids were intermediate to the DNA amounts of their respective parental species. Price *et al.* (1983), analysing the inheritance pattern of genome size in the genus *Microseris*, observed a different phenomenon. When two species which differed by 10 per cent in genome size were crossed, the genome sizes of the resulting F_1 progeny did not cluster around the parental midpoint. Instead, hybrids were observed with nuclear DNA amounts that differed significantly from the parental midpoint. Price (1988) suggested that some portions of the DNA are unstable in various F_1 hybrids and can randomly fluctuate in these hybrids.

Due to both the conflicting observations with respect to the inheritance of genome size in plants, and the potential adaptive significance of genome size in maize, more information is needed on the nuclear DNA content of F_1 hybrids in maize. The objectives of this study were to determine whether the nuclear DNA contents of certain F_1 hybrids in maize are variable and

to determine if the nuclear DNA contents in F_1 hybrids are equivalent to their respective parental midpoint.

Materials and methods

Experiment one

Initially, 10 inbred lines (see Table 1) were screened concerning their nuclear DNA amount. The lines were selected on the basis of their diverse pedigrees. From these, the two inbreds with the largest genome sizes, A619 and H99, and the two with the smallest genome size, R53 and I205, were selected. These lines were then grown at the University of Illinois Agronomy South Farm where they were crossed in all combinations in both 1989 and 1990. At least three crosses were made per hybrid. The inbred lines were also selfed in order to maintain the lines. The increased seed of each inbred line, as well as the hybrids, were analysed to determine their genome size.

Nuclei were isolated from the stems of individual 2-week-old seedlings, stained with the fluorochrome DAPI (4′-6-diamidino-2-phenylindole), and analysed flow cytometrically using the methods of Rayburn *et al.* (1989). Five thousand nuclei were examined per isolation.

Initially, each of the 10 inbred lines was analysed. Nuclear suspensions were obtained from individual plants. Ten plants were analysed for each inbred line with Va35 as an external standard. Data were collected as fluorescence intensity relative to Va35. Va35 was defined as having 100 Arbitrary Units (A.U.). The data were converted to picograms (pg) on the basis of Va35 having 10.0 pg per 4C nucleus (Rayburn *et al.*, 1989).

The selfed seeds of each inbred line and 12 hybrids were then analysed with R53 as an external standard and R53 defined as having a genome size of 100 A.U.

Table 1 Genome size data in 10 maize inbred lines

Inbred line	DNA amounts* (A.U.)	Standard deviation	Picograms per 4C nucleus
A619	107.6	1.1	10.8
B14	104.2	3.8	10.4
H55	104.7	2.2	10.5
H98	101.3	2.5	10.1
H99	107.3	1.5	10.7
I205	96.3	0.7	9.6
Ms92	102.0	0.9	10.2
NC250	102.5	2.1	10.3
R225	105.1	0.9	10.5
R53	96.7	1.3	9.7

*Based on Va35 = 100 A.U.

All data were collected as fluorescence intensity relative to R53. The number of plants per line or hybrid examined is listed in Table 2.

Statistical analyses were run to determine if significant differences among the lines were observed. To determine if genome size was inherited in a simple additive manner, a *t*-test was run between the theoretical and observed genome size of each hybrid. The theoretical genome size was determined as $(P1 + P2)/2$, where $P1$ and $P2$ are the values observed for the two parental inbred lines.

Table 2 Genome sizes of four inbred lines and their respective F_1 hybrids

Line	Number of plants examined	DNA amount* (A.U.)	Standard deviation
Inbreds			
R53	26	100.0	2.0
H99	8	109.1	3.2
A619	4	109.3	2.8
I205	5	101.2	2.8
Hybrids			
A619 × I205	7	103.1	2.9
I205 × A619	11	106.3	2.5
A619 × R53	12	102.4	4.4
R53 × A619	18	102.4	2.9
H99 × I205	11	104.7	4.1
I205 × H99	5	104.1	4.1
H99 × R53	18	101.8	2.0
R53 × H99	5	103.8	2.1
A619 × H99	5	108.7	4.6
H99 × A619	5	108.0	2.4
I205 × R53	5	103.1	2.7
R53 × I205	5	100.2	2.0

*Based on R53 = 100 A.U.

Table 3 DNA amount in specific crosses of three F_1 hybrids

Hybrid	Year	Cross	Number of plants	Mean	Standard deviation
H99 × R53	1989	A	4	100.8	2.6
	1989	B	6	101.9	1.6
	1990	A	8	102.2	2.1
R53 × A619	1989	A	6	100.9	2.9
	1989	B	6	104.4	2.3
	1990	A	6	102.0	2.7
I205 × A619	1989	A	5	105.8	3.3
	1990	A	6	106.6	2.0

Experiment two

Nine inbred lines and 14 hybrids were analysed in this experiment (see Table 3). The hybrids were selected on the basis of their heterotic responses in the field according to Zanoni & Dudley (1989). The hybrids selected represented the widest range of heterotic responses observed in 91 F_1 hybrids examined by Zanoni & Dudley. At the time the experiment started, no information concerning the genome size of the hybrids was known. The nine inbred lines selected are the parental lines of the 14 F_1 hybrids. The parental lines were grown on Agronomy South Farm; selfed and F_1 hybrid seeds were obtained in the summer of 1990. At least three crosses were made per hybrid.

The nuclear DNA content of the inbreds and F_1 hybrids was determined as previously described except two plants were combined per nuclear isolation and W22 was the inbred chosen for the external standard. W22 was therefore defined as having 100 A.U. *t*-tests were then run between the theoretical and observed genome size of the F_1 hybrids.

Results

Experiment one

The genome sizes of the 10 inbred lines are listed in Table 1. Approximately an 11 per cent difference in genome size among these lines was observed. The DNA contents ranged from 5.4 pg per 2C nucleus in A619 to 4.8 pg per 2C nucleus in I205. Two of the lines had a fairly high genome size (A619 — 5.4 pg; H99 — 5.35 pg) while two of the lines had smaller genome sizes (R53 — 4.85 pg; I205 — 4.8 pg). The remaining six inbred lines all had genome sizes around 5.15 pg per 2C nucleus.

The data listed in Table 2 were taken from progeny produced at the University of Illinois Agronomy farm. A 9.3 per cent difference between the extremes in genome size was noted. A619 was observed to have the largest genome size with 109.3 A.U., while R53 was observed to have the smallest with 100 A.U. Both R53 and I205 were observed to have small genome sizes while A619 and H99 had large genome sizes.

A paired *t*-test between the observed genome size and its theoretical genome size was run for each hybrid. No significant difference between the observed and calculated genome size was observed. In addition, little variation was noted between crosses or between years within specific crosses (Table 3).

Upon observing the distribution of the F_1 hybrid plants within individual crosses, two distinct patterns were noted. In the first pattern, the F_1 hybrids clustered around the parental mean (Fig. 1). In this pattern the majority of plants had a nuclear DNA content approximately equal to the parental mean. A few plants were observed, however, that had DNA amounts both higher and lower than the parental mean. In the second pattern, the nuclear DNA amount varied a great deal with the majority of plants not having the parental mean DNA amount (Fig. 2). The plants could cluster around one parental DNA amount or could vary from one parental DNA amount to another with no real clustering of the F_1 plants. No clear correlation

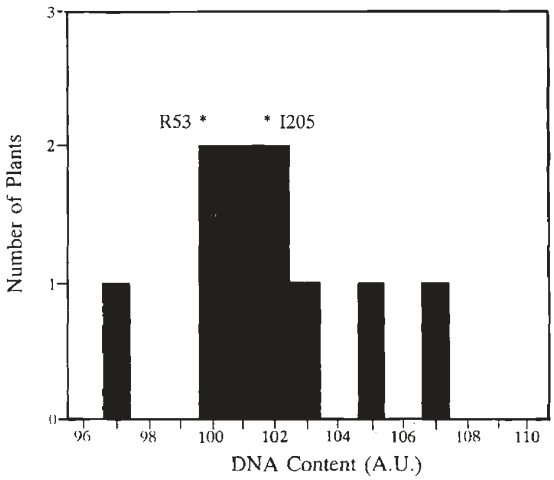

Fig. 1 Histogram of R53 × I205 F_1 hybrids. Both crosses, R53 (female) × I205 (male) and its reciprocal cross were combined. The hybrid mean was 101.6 A.U. The majority of the progeny are clustered around the mid-parent (the expected F_1 mean).

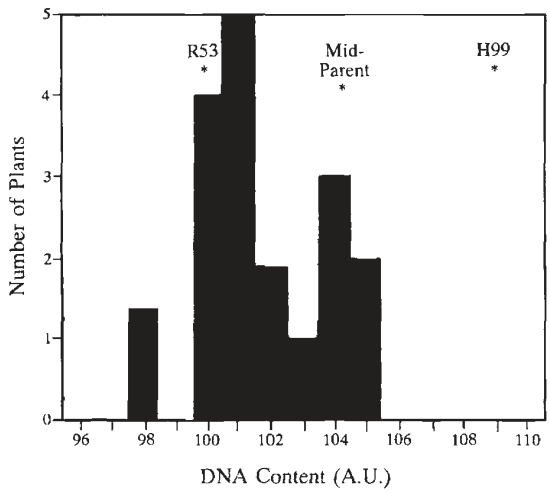

Fig. 2 Histogram of the specific cross H99 (female) × R53 (male). The hybrid mean was 101.8 A.U. The progeny appear to cluster around the low parent as well as the mid-parent.

between pattern and parental lines was observed. In addition, no correlation between year grown and pattern was observed.

Experiment two

The nuclear DNA amounts of the parental lines and the F_1 hybrids are listed in Table 4. The 2C nuclear DNA amount ranged from 5.3 pg in Pa91 to ≈4.8 pg in Mo17. The majority of inbred lines had a 2C nuclear DNA amount of ≈ 5.0 pg.

The 2C nuclear DNA amount ranged from ≈ 5.4 pg in H102 × H100 to ≈ 5.0 pg in both H100 × B84 and B84 × Mo17 (see Table 3). In nine of the 14 hybrids, the nuclear DNA content did not significantly differ from their parental means. In seven of these cases, the genome size of the hybrid was higher than its parental mean. In five of the 14 hybrids, the nuclear DNA content was significantly higher than their respective parental means. H102 × H100 deviated the most from the expected F_1 mean with a 5.5 per cent increase in DNA. The standard deviations for the F_1 hybrids were low ranging from 0.3 to 2.6. The 2.6 standard deviation was observed in two hybrids. Excluding these hybrids, the standard deviation within F_1 hybrids ranged from 0.3 to 1.8. All the F_1 hybrid plants cluster around their respective F_1 means (Figs 3 and 4).

Discussion

Experiment one

That a significant difference was observed among the 10 inbred lines is not surprising. Rayburn *et al.* (1985) observed a 16 per cent variation among 18 U.S. inbred lines. The 11 per cent variation observed here is well

Table 4 Genome size of nine inbred lines and specific F_1 hybrids

Line	Mean genome* size (A.U.)	Standard deviation	Picograms of DNA per 4C nucleus
Inbreds†			
Pa91	99.2	3.6	10.6
Va26	96.7	2.8	10.3
H102	95.6	3.8	10.2
H95	95.4	1.3	10.2
H100	94.4	1.2	10.1
N7A	92.9	3.2	9.9
B84	92.7	2.6	9.9
B73	90.8	3.6	9.7
Mo17	88.8	4.0	9.5
Hybrids			
H102 × H100	100.2‡	0.3	10.7
H95 × Va26	99.4‡	0.8	10.6
H100 × N7A	98.7‡	1.8	10.6
Pa91 × H102	98.4	2.6	10.5
Pa91 × H100	98.2	0.8	10.5
H95 × B84	97.4‡	1.1	10.4
H102 × Mo17	97.3‡	1.0	10.4
H95 × B73	96.1	1.9	10.3
Va26 × Pa91	95.3	0.5	10.2
Va26 × N7A	95.2	0.3	10.2
B73 × B84	94.4	2.6	10.1
H100 × B73	93.9	1.1	10.1
H100 × B84	92.6	1.2	9.9
B84 × Mo17	92.0	0.6	9.9

*Based on W22 = 100 A.U.
†Six samples, each sample consisting of nuclei from two plants, were analysed for each inbred line and hybrid.
‡Denotes significant deviation from the expected F_1 mean at $t = 0.05$ level, sign considered.

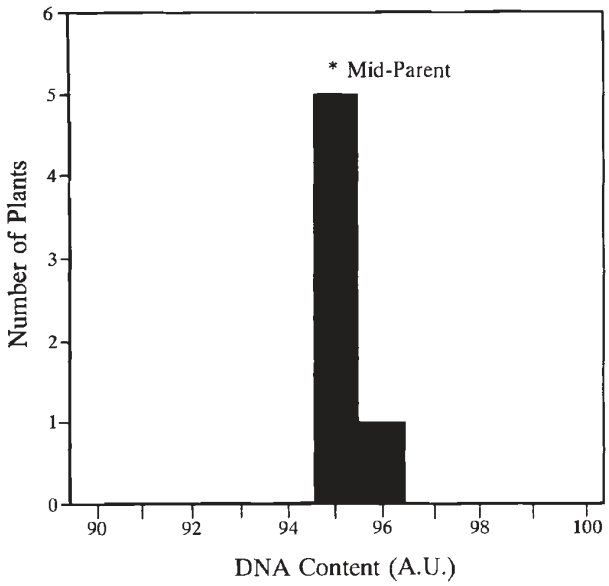

Fig. 3 Histogram of Va26 × N7A F_1 hybrid progeny. The hybrid mean was 95.2 A.U. The progeny are clustered around the mid-parent.

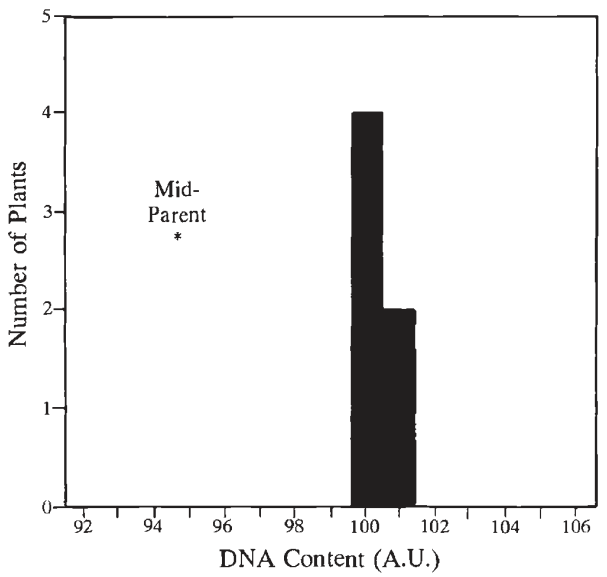

Fig. 4 Histogram of H102 × H100 F_1 hybrid progeny. The hybrid mean was 100.2 A.U. The progeny cluster around a mean larger than either parental DNA amount.

within the expected variation. R53 and I205 have the smallest genome size of any inbred line examined to date. The largest genome size observed in this study was approximately 8 per cent smaller than the largest genome size reported by Rayburn *et al.* (1985). That these inbreds did not encompass the whole range of genome sizes of U.S. inbred lines is not unexpected. Rayburn *et al.* (1985) chose maize lines that originated from the northen and southern latitudes of the U.S. as well as the midwest. All of the lines chosen for this study are midwestern U.S. cornbelt inbred lines. The 11 per cent variation in genome size observed in this particular study indicates the heterogeneity of the cornbelt inbred lines. An interesting observation is that the genome sizes of R53 and I205 are lower than the genome size of any other maize line examined to date, with the exception of Gaspe flint.

Bennett (1972) proposed that genome size and minimum generation time were correlated in plants. Rayburn *et al.* (1985) suggested that the small genome sizes of certain northern maize populations was due, at least in part, to selection for rapid maturation in the more time limited northern environment. This hypothesis indicates a relationship between flowering time and genome size in maize. The flowering times of R53 and I205 are not that dissimilar from other corn belt maize. R53 flowers approximately 11 days earlier than I205 at Urbana, IL. In fact, I205 has one of the longest days to flowering of the 10 inbred lines examined in this study. The inbreds with the largest genome size, H99 and A619, both flower about 5 days earlier than I205. Note, however, that the range in flowering times of the 10 inbred lines was only 15 days. The possibility exists that flowering time is indeed correlated with genome size but the differences in flowering time observed in this study may not be large enough to induce a detectable difference in genome size.

Within each inbred line, the nuclear DNA amount was stably inherited. Had the nuclear DNA amount been found to vary from generation to generation within these inbred lines, making hybrids and analysing them for nuclear DNA amount would have provided no useful information.

The nuclear DNA amounts of the F_1 hybrids did not statistically deviate from their respective parental mid-point at $P > 0.05$ level. At first glance, these results appear to support the hypothesis of Hutchinson *et al.* (1979) which would indicate that the nuclear DNA amount of the hybrids should be equivalent to its parental mean nuclear DNA amount. However, upon closer examination of the data, the inheritance of nuclear DNA content appears more complex. When one examines the nuclear DNA amount in individual plants of specific crosses, two types of DNA inheritance patterns are observed. The data presented in Fig. 1 support a simple inheritance of DNA amount. Most of the plants of this hybrid had a nuclear DNA amount approximately equal to the parental mid-point. The data presented in Fig. 2, however, are contrary to the simple inheritance model. The majority of the F_1

hybrids in this cross had a genome size lower than the parental midpoint. In addition, the nuclear DNA amount of the individual plants did not appear to be the same. Instead of all the F_1 plants clustering around a single mean, plants were observed to have different DNA contents.

To ensure that contamination was not responsible for the observed data, three different crosses, which were made over a 2-year period, were analysed. In all cases, the mean nuclear DNA amount of each cross was nearly identical to the overall mean of the F_1 hybrid (Table 3). In addition the DNA content variation among individual plants within each cross of a specific hybrid was similar indicating that the DNA content variation among individual plants within a hybrid is real and reproducible. These results are very similar to the results of Price *et al.* (1983). The nuclear DNA amount of specific F_1 plants appears to be unstable. In certain F_1 hybrids, the variation in DNA amount may be as much as 8 per cent, approximately the same DNA variation observed between the two parental lines involved in the cross.

Experiment two

The F_1 hybrids to be used for this experiment were selected on the basis of their heterotic responses, which had been determined previously by Zanoni & Dudley (1989). The parental lines of the hybrids chosen were Stiff Stalk (S) types and Lancaster (L) types. The hybrids chosen had both low and high heterotic responses. The hybrids represented S × S, L × L, and S × L types. It was important to this study that no information on genome size was available. Therefore, there was no bias with respect to genome size in selecting the parental lines or their hybrids.

The range of genome sizes observed in the inbred lines was similar to that observed in experiment one. Four of the five L types were observed to have the largest genome sizes. Mo17 was the lone L type that had a low genome size. In fact, Mo17 had the lowest genome size observed in this study. The four S types all had similar genome sizes. The indications are that S types, for the most part, have a smaller genome size than L types. To substantiate this, however, more S and L lines need to be examined.

That five of the F_1 hybrids had a statistically higher genome size than their respective parental means was unexpected. In experiment one, while genome size was variable among F_1 plants within specific crosses, if the DNA amount of a plant varied, it was usually lower than the parental mean and in several cases appeared smaller than the lowest parental line. In the hybrids examined in experiment two, however, F_1 hybrid plants within each cross appeared to have the same genome size. There was little plant-to-plant variation within these crosses. In addition, the mean genome size of 12 of the 14 hybrids was numerically higher than their respective parental means. These trends are opposite to the trends in experiment one.

The differences in genome size stability between the two experiments may be due to how the material was selected. In experiment one there was no concern as to the agronomic performance of the F_1 hybrid. The lines were selected totally on the nuclear DNA content variation among the lines. In experiment two, the selection of the lines was based totally on agronomic performance. Individual plants within a hybrid might therefore be expected to be stable. If F_1 plants were highly variable for DNA amount, they might also be variable for other characters as well. As the hybrids observed in this experiment were very uniform in all their characteristics, it is not surprising that their genome size was uniform as well. Note that the uniformity of the hybrids in experiment one was not examined.

Conclusions

The results of both experiments indicate that the nuclear DNA content is not simply inherited in F_1 hybrids in maize. In two previous studies (Baer & Schrader, 1985; Michaelson *et al.*, 1991), it has been reported that the genome size of F_1 hybrids of maize is intermediate between their respective parental genome sizes. In both of these studies, only a few parental combinations were examined. As evident from this study, some parental combinations result in F_1 hybrids with genome sizes equal to their respective parental means. The hybrids examined in the previously reported studies could be the result of such hybrids. In addition, a large number of progeny was not examined in these studies. In Baer & Schrader (1985) only one F_1 plant was examined, while in Michaelson *et al.* (1991) no indication as to how many F_1 plants were examined was given. As observed in the current study, the nuclear DNA content variation within a cross may be such that a large number of individual plants must be examined to gain an accurate estimation of a mean genome size of a particular set of F_1 hybrids.

Price (1988), summarizing the results of interspecific crosses in *Microseris*, hypothesized that DNA sequences, which account for DNA content differences among F_1 plants, are unstable and undergo deletion or amplification in specific F_1 plants. Appearances dictate that the same phenomenon occurs in intraspecific crosses in *Zea mays*. These data also support the contention by Flavell (1982) that the DNA within plants is

not stagnant but undergoes amplification, deletions, rearrangements and translocations, all of which are designated macromutations. These macromutations can cause certain segments of the genome to change rapidly. Evidence of such changes in F_1 hybrids was observed by Rogers and Bendich (1987).

The plant genome can, therefore, be fluid with respect to DNA sequence copy number. In maize, whether DNA sequence copy numbers are stable or not depends on the parental inbred lines used in each cross. This flexibility could be of tremendous importance to the role of nucleotypic selection in the evolutionary adaptation of maize.

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