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Effects of Polyploidy on Photosynthetic Rates, Photosynthetic Enzymes, Contents of DNA, Chlorophyll, and Sizes and Numbers of Photosynthetic Cells in the C_4 Dicot *Atriplex confertifolia*[1]

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ABSTRACT

Photosynthetic rates, chlorophyll content, and activities of several photosynthetic enzymes were determined per cell, per unit DNA, and per unit leaf area in five ploidal levels of the C_4 dicot *Atriplex confertifolia*. Volumes of bundle sheath and mesophyll protoplasts were measured in enzymatic digestions of leaf tissue. Photosynthetic rates per cell, contents of DNA per cell, and activities of the bundle sheath enzymes ribulose 1,5-bisphosphate carboxylase (RuBPC) and NAD-malic enzyme per cell were correlated with ploidal level at 99% or 95% confidence levels, and the results suggested a near proportional relationship between gene dosage and gene products. There was also a high correlation between volume of mesophyll and bundle sheath cells and the ploidal level. Contents of DNA per cell, activity of RuBPC per cell, and volumes of cells were correlated with photosynthetic rate per cell at the 95% confidence level. The mesophyll cells did not respond to changes in ploidy like the bundle sheath cells. In the mesophyll cells the chlorophyll content per cell was constant at different ploidal levels, there was less increase in cell volume than in bundle sheath cells with an increase in ploidy, and there was not a significant correlation (at 95% level) of phosphoenolpyruvate carboxylase activity or content and pyruvate,Pi dikinase activity with increase in ploidy. The number of photosynthetic cells per unit leaf area progressively decreased with increasing ploidy from diploid to hexaploid, but thereafter remained constant in octaploid and decaploid plants. Numbers of cells per leaf area were not correlated with cell volumes. The mean photosynthetic rates per unit leaf area were lowest in the diploid, similar in 4×, 6×, and 8×, and highest in the decaploid. The photosynthetic rate per leaf area was highly correlated with the DNA content per leaf area.

Polyploidy often produces immediate and dramatic changes in photosynthetic rates of plants. Photosynthetic carbon uptake increases in higher ploidal levels on a leaf area basis in *Agropyron cristatum* (L.) (7), *Festuca arundinacea* (Schreb.) (9), and *Panicum virgatum* (22). It increases on a Chl basis in *Lolium perenne* (L.) (13), and per cell in *Medicago sativa* (L.) (10), *Panicum virgatum* (22), and *Pennisetum americanum* (23). Photosynthetic rates per leaf area decrease or do not change in other polyploid plants such as *Triticum* and *Aegilops* species (2), *Datura stramonium* (L.) (6), *Lycopersicon esculentum* (L.) (1), and *Ricinus communis* (L.) (18).

Polyploidy has interrelated effects on structural, biochemical, and physiological elements, and this may influence photosynthetic rates (1,2,4,6,9,10,13,22,23). The number of nuclear chromosomes determines, to some extent, the size of leaves, the size of cells, the number of chloroplasts per cell, and amounts of photosynthetic enzymes and pigments in cells of polyploid plants. Because of the interactions of these various factors, the basis of expression of photosynthetic measurements is critical for understanding the effects of polyploidy on photosynthesis. The commonly used bases of Chl and protein are not informative if one does not know how amounts of these compounds change in relation to the number of chromosomes. Measurements based on leaf area are not useful without knowing the number of cells (and thus the number of nuclear genomes) in a unit of leaf area. When the number of cells per unit leaf area is known, then photosynthetic rates can be expressed per cell. Since the amount of nuclear DNA and the size of cells are two of the factors most affected by changes in ploidy, the most accurate description of changes in photosynthesis in polyploid plants comes from measurements expressed per cell and per unit DNA.

With an interest in the effects of polyploidy on C_4 photosynthesis, we found in two polyploid species of the Gramineae a proportional relationship between gene dosage and gene products. However, there were different consequences on photosynthesis per unit cell volume and per unit leaf area which may be related to the origin of the polyploidy (22,23).

Atriplex confertifolia (shadscale) of the family Chenopodiaceae is a naturally occurring polyploid complex that exhibits a complete range of even-numbered ploidal levels from diploid (2×) to decaploid (10×). Polyploid races of this species occupy the sites of former Pleistocene lakes, and have, therefore, only appeared in the last 10 to 15 thousand years (17). We have used five chromosome races of *A. confertifolia* to determine photosynthetic rates expressed on cell, DNA, and leaf area bases, and also the relationships of photosynthetic rates to changes in cell size and number, activities and amounts of photosynthetic enzymes, and amounts of DNA and Chl. These five ploidal levels allow us to determine whether the effects of polyploidy are constant across all

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ploidal levels, and if there is an optimum ploidal level for photosynthesis.

MATERIALS AND METHODS

Plant Material

Seeds of polyploid races of *Atriplex confertifolia* (Torr. and Frem.) S. Wats. (shadscale) were obtained from Dr. Stewart Sanderson of the Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602. To improve germination, utricles (fruits) were gently ground with a mortar and pestle to break up the pericarps. The "naked" seeds obtained were soaked for 20 min in 20% (v/v) ethanol, rinsed with H_2O, then planted in a 3:1 mixture of commercial potting soil and sand. "Gibrel" germination stimulator mixed with H_2O was applied to the surface of the potting medium. Seedlings were produced from the following populations: 2× Rush Valley, Tooele Co., Utah; 4× Lockes, Nye Co., Nevada; 4× Faust, Tooele Co., Utah; 6× Cortez, Colorado; 8× Faust, Tooele Co., Utah; 8× Sevier Lake, Millard Co., Utah; and 10× Eskdale, Millard Co., Utah (17). Photosynthesis was not measured in plants from 4× Lockes or 8× Faust populations. Other data for 4× and 8× ploidal levels are means of two or three replicates for each of the two populations.

Ploidal levels were confirmed by counting chromosomes in mitotic cells of root tips that were treated with 2 mM 8-hydroxyquinoline for 5 h at 10°C, fixed in 100% ethanol:glacial acetic acid (3:1, v/v), digested 1 h at room temperature with snail glusulase enzyme, and stained with 1% acetocarmine (16). Plants of known chromosome number were propagated by stem cuttings rooted in potting soil (14). Plants were subsequently grown in a 1:1 mixture of potting soil and sand, except for hexaploid plants which were grown in 3:1 soil to sand. Plants were grown in a controlled-environment room under 500 μmol quanta$\cdot m^{-2}\cdot s^{-1}$ (400–700 nm) irradiance at the plant level, provided by a combination of 440 w sodium vapor and metal halide lamps, with a 13 h photoperiod. Air temperature was 30°C during the light period and 19°C in the dark. Plants were fertilized once a week with a Hoagland solution (23) and watered between feedings as necessary. Hexaploid plants grown in the growth room often had tougher and less succulent leaves and much shorter internodes (suggesting less than optimum growth conditions) compared to plants with the other ploidal levels and compared to 6× plants grown in a greenhouse. Measurements of carbon assimilation rates and DNA content were made on greenhouse grown 6× plants.

CO_2-Exchange and DNA Assay

The apical 4 cm of shoots were used in gas exchange studies. Twenty-four h prior to gas exchange measurements, older leaves 4 to 6 cm from the apex, axial branches and the apical whorl were removed from shoots. For measurements, the shoot was mounted in a 4 × 4 cm wooden frame with the leaves held flat and perpendicular to the light source with nylon fishing line. The frame holding the shoot was inserted into a 5.1 × 8.9 × 1.5 cm glass cuvette (Bingham Interspace, Hyde Park, Utah). Steady-state CO_2 uptake was measured with an Analytical Development Co. infrared gas analyser (225·MK3) controlled by a Bingham Interspace model BI-2 controller system. This was an open system and was operated in differential mode. CO_2 uptake was measured at a leaf temperature of 30°C, and an irradiance of 2000 μmol quanta$\cdot m^{-2}\cdot s^{-1}$ (400–700 nm). Ambient [CO_2] was 330 to 340 $\mu L\cdot L^{-1}$, and flow rate in the cuvette was 0.3 to 0.4 $L\cdot min^{-1}$. Five to nine leaves with a total area of 3 to 6 cm^2 were enclosed in the cuvette. Results are means of three replicate measurements on different branches of one or two different plants.

Following CO_2 exchange measurements, leaves were cut from the stem, and leaf area was measured with a Li-Cor Li-3000 leaf area meter. The leaves were frozen and stored in liquid N_2 until use. Frozen leaves were ground in a mortar and pestle with 5 volumes of 25 mM Hepes-KOH (pH 7.5), 5 mM DTT, and 10 mM $MgCl_2$. The final volume of the homogenate was measured; 40 μL aliquots were taken for Chl measurements, and 100 μL of the homogenate was diluted with 1.0 mL of 11 mM Tris-HCl (pH 7.0), 11 mM EDTA-Na_2, and 2.2 M NaCl. This latter sample was used for measurement of DNA according to Baer *et al.* (3). DNA assays were performed in duplicate.

Chl per Unit Leaf Area

Discs 4.0 mm in diameter were punched from the center of the leaves with a cork borer, weighed, and placed in a test tube. The discs were frozen with liquid N_2, and ground to powder with a glass rod. One mL of 100% ethanol was added to each disc. Chl was extracted for 24 h at 5°C in the dark. Measurements were performed in triplicate for each ploidal level. Chl concentrations of the extracts were calculated using the equations of Wintermans and De Mots (24).

Cell Number per Unit Leaf Area

Leaf discs 4.0 mm in diameter were punched out with a cork borer. One disc was placed in a 1.5 mL centrifuge tube with 1.0 mL of 5% (w/v) chromium trioxide prepared in 1 N HCl. Tubes were placed in the dark for 7 d at room temperature. Digested discs were disrupted by vortexing. Cells were counted in a 0.2 mm depth hemocytometer using a phase-contrast microscope with either a 10× or 20× objective. Counts were made on triplicate samples for each ploidal level.

Enzyme Assays

Leaves were harvested under growth-room light and frozen in liquid N_2. Frozen leaves (0.1–0.4 g fresh weight) were ground with a mortar and pestle in 5 volumes of 100 mM Hepes-KOH (pH 7.5), 10 mM $MgCl_2$, 4 mM $MnCl_2$, 10 mM DTT, 1 mM EDTA, 2.5 mM Na-pyruvate (for stabilization of PPDK[3]), 80 μM pyridoxal phosphate, 0.5% (w/v) BSA, 0.05% (v/v) Triton X-100, and 1.5% (w/v) soluble PVP. When the crude homogenate was centrifuged, RuBPC activity sedimented (possibly binding to membranes for unknown rea-

[3] Abbreviations: PPDK, pyruvate, orthophosphate dikinase; NAD-ME, NAD-malic enzyme; PEPC, phospho*enol*pyruvate carboxylase; RuBPC, ribulose 1,5-bisphosphate carboxylase; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase.

sons), which may explain previously reported lack of activity in this species (21). Thus, the crude extract was used directly without any filtration or centrifugation for assay of RuBPC activity. RuBPC was assayed by incorporation of $^{14}CO_2$-radioactivity into acid-stable products (11). Other enzymes were assayed spectrophotometrically at 340 nm using the supernatant fraction following centrifugation of the crude homogenate at 10,000*g* for 4 min. For NAD-ME, extracts were desalted by centrifugation through Sephadex G-25 equilibrated with grinding medium from which pyruvate, Triton, PVP, and pyridoxal phosphate were omitted. The desalted extracts were incubated under N_2 gas for 1 h at room temperature prior to assay.

PPDK and PEPC were assayed as in Warner and Edwards (23) and NAD-ME according to Hatch *et al.* (8). Assays were performed at 30°C in a 1.0 mL reaction volume.

Chl was measured in 96% (v/v) ethanol extracts of the crude homogenates (24).

Purification of PEPC and Antibody Production

PEPC was purified according to the procedure of Uedan and Sugiyama (19). Fully expanded leaves were harvested under full irradiance from hexaploid plants in both the growth room and the greenhouse. Extraction and ion-exchange chromatography buffers contained 10% and 5% (v/v) glycerol, respectively. Proteins were eluted from the DEAE-cellulose anion-exchange column with a linear gradient of 10 to 300 mM KCl. PEPC eluted in two peaks at about 20 mM (peak I) and 80 mM (peak II) KCl. Active fractions for each of the two forms of the enzyme were pooled separately. The enzyme was precipitated with 60% ammonium sulfate, and resuspended in, and dialysed against, column buffer. The partially purified protein solution was brought to 30% (v/v) glycerol and stored at 5°C.

Peak II protein appeared more pure than peak I on SDS-PAGE (>90%), so this protein was used for the production of anti-PEPC antibodies in chicken. To ensure antigenic purity, about 1.0 mg of peak II protein was subjected to nondenaturing PAGE. The band indicated by Coomassie blue and activity staining (15) of companion lanes was cut out and emulsified in 1 mL of 10 mM Hepes-KOH (pH 7.0). The protein contained in the emulsified gel had a subunit mol wt of 100,000 on SDS-PAGE. Gel slurry containing about 225 μg of protein was mixed 1:1 with Freund's complete adjuvant, and injected into a laying hen in both sides of the breast and in both thighs. A booster injection with Freund's incomplete adjuvant was given after 7 d.

Three weeks after the booster injection, antibodies were purified from yolks of the hen's eggs according to Polson and von Wechmar (12). Immunoglobulin G was also isolated from eggs laid before the injections were given (preimmune eggs).

Antibody-antigen reactions were tested in double-diffusion Ouchterlony gel experiments. Optimum precipitin lines were obtained when the antibody solution contained 6 mg protein·mL^{-1}, and the antigen solutions contained 0.25 to 1.0 mg protein·mL^{-1}.

The PEPC from peak I and II were antigenically identical, but the antibody reacted more strongly with peak II enzyme. Therefore, the total quantity of the enzyme based on rocket immunoelectrophoresis may be underestimated. The antibody reacted with crude extracts from leaves of all ploidal levels and no spur was produced. This indicates that the enzyme was immunologically the same in all ploidal levels. The anti-*A. confertifolia* PEPC antibody did not react with *A. confertifolia* root extract, with extracts from bundle sheath cells as prepared below, nor with BSA. Immunoglobin G from preimmune eggs did not react with purified *A. confertifolia* PEPC, nor with crude leaf extracts.

Enzymatic Isolation of Protoplasts (to Determine Cell Volume) and Cells (to Determine PEPC Protein per Cell)

Enzymatic digestion of young, fully expanded leaves yielded mostly intact cells with about 10% intact protoplasts. Diameters of protoplasts were measured with an ocular micrometer on a phase-contrast microscope. Mesophyll and bundle sheath cells were separated by differential centrifugation and centrifugation on sucrose/dextran step gradients. Chl and numbers of cells were determined in the final cell suspensions.

Isolated cells were subjected to two freeze-thaw cycles and ground in a glass homogenizer. Chl was measured in aliquots of the cell homogenate. For determination of PEPC amount per cell, the cell extracts were centrifuged at 10,000*g* for 4 min, and the supernatant was used for rocket immunoelectrophoresis assay. Dilutions of PEPC peak II protein were used to establish a standard curve. Ten μL of sample or standard solution were loaded per well. Increase in peak height was linear from 60 to 475 ng protein. Protein was measured by the Bradford (5) method, using fatty-acid-free BSA as a standard.

Data Analysis

Data were analyzed by linear regression. For $n = 5$ samples (ploidal levels), significance at the 5% and 1% levels occurred at correlation coefficient values of 0.878 and 0.959, respectively. Regression lines and correlation coefficients are given in the figures when significant correlation occurs at ≥95% confidence levels. Note that regressions are made with fixed increments of ploidy, *i.e.* ploidy is not a measured variable.

RESULTS

Measurements Expressed Per Cell

The variations, in a number of parameters, expressed on a per cell basis versus ploidal level in *A. confertifolia*, are shown in Figure 1. The solid lines are linear regressions on the data, while the *broken lines* are drawn from the origin to intercept the linear regression line at the 6× ploidal level. Linear regressions in most cases had a close fit to the *broken line*, which suggests a doubling of the measured parameter with a doubling in ploidy.

The total DNA content of the leaf expressed per photosynthetic cell was correlated with ploidal level ($r = 0.943$, $P < 0.05$), although the contents at the 6× and 8× levels were similar (Fig. 1A).

Photosynthetic rate per cell was highly correlated ($r = 0.965$, $P < 0.01$) with ploidal level (solid line, Fig. 1B), although the rates were similar for 6× and 8× plants.

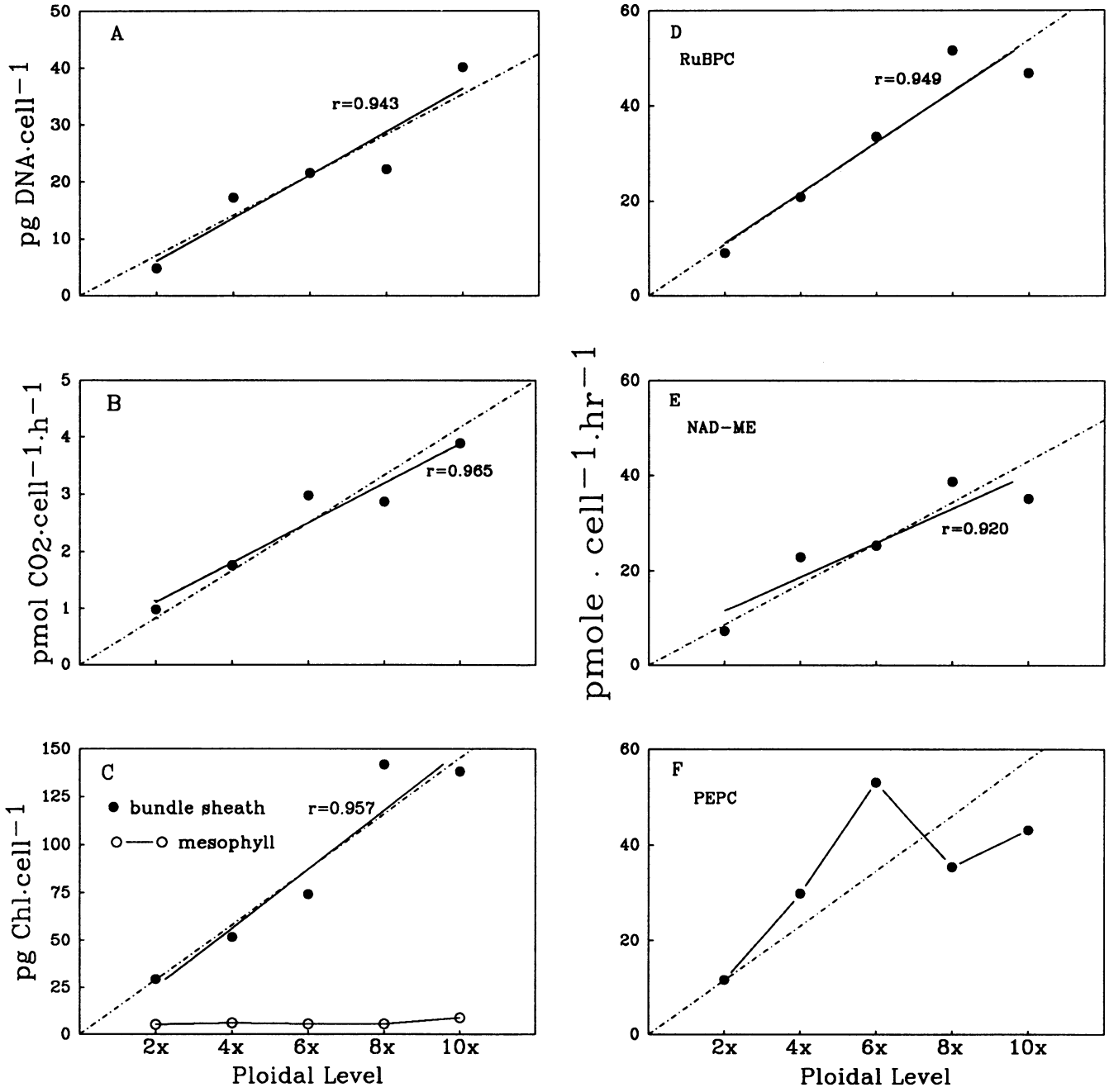

Figure 1. A, The total DNA content of leaves expressed per photosynthetic cell in *A. confertifolia*. Note this is based on total leaf DNA and is not an absolute measure of DNA per photosynthetic cell. It is taken to indicate the relative differences in DNA content per photosynthetic cell between the different ploidal levels. Calculated from data in Figures 4C and 5. Standard deviations calculated by addition in quadrature of the independent standard deviations of the original measurements were less than the size of the graph symbols. *Solid lines* are regression analyses. *Broken lines* are arbitrarily drawn from the origin through the regression lines at 6× (representing a theoretical doubling of function with doubling of ploidy). B, Photosynthetic rate per cell in leaves of *A. confertifolia* calculated from data in Figures 4A and 5. The photosynthetic rate of 6× plants from the growth chamber was 1.73 pmol $CO_2 \cdot cell^{-1} \cdot h^{-1}$. Standard deviations determined as in A were less than the size of the graph symbols. C, Chl per cell in leaves of *A. confertifolia*. Numbers of cells and Chl were determined in suspensions of purified mesophyll and bundle sheath cells obtained by enzymatic digestion of leaves. D, E, and F, Activities of RuBPC, NAD-ME, and PEPC on a cell basis. Enzyme activities were measured per unit leaf area, and calculated per cell (bundle sheath for RuBPC and NAD-ME, and mesophyll for PEPC) in units of pmol activity $cell^{-1}$ h^{-1} based on data in Figure 4.

Chl per bundle sheath cell increased linearly with ploidal level ($r = 0.957$, $P < 0.05$) but was similar at the 8× and 10× levels (Fig. 1C). Chl per mesophyll cell was nearly constant at all ploidal levels, with a slight increase at 10×.

Activities of RuBPC and NAD-ME per cell were correlated with ploidal level at a 95% confidence level ($r = 0.949$ and 0.920, respectively, Fig 1, D and E), although activities were lower in 10× than in 8× cells. Activity (Fig. 1F, $r = 0.699$) and content ($r = 0.337$, $P > 0.05$, data not shown) of PEPC per cell were not significantly correlated with ploidal level, nor was the activity of PPDK ($r = 0.780$, $P > 0.05$, data not shown). As DNA per cell, Rubisco activity per cell, and photosynthesis per cell, were all highly correlated with ploidal level (Fig. 1), there was a significant correlation of DNA cell^{-1} and Rubisco activity cell^{-1} with photosynthesis rate cell^{-1} (Table I). The rate of CO_2 fixation mg DNA^{-1} was also highly correlated with Rubisco and NAD-ME activity mg DNA^{-1} but not with PEP carboxylase activity and content (Table I).

The volume of bundle sheath cells increased, and was correlated with ploidal level (Figs. 2 and 3, $P < 0.01$). While the increase in volume of mesophyll cells was less (about 1.4 fold with doubling of ploidy, according to linear regression) than that in bundle sheath cells, mesophyll cell volume was also correlated with ploidal level ($P < 0.05$). There was a strong correlation between RuBPC cell^{-1} with bundle sheath cell volume but not of PEPC cell^{-1} with mesophyll cell volume. The increase in mesophyll and bundle sheath cell volumes were correlated with CO_2 fixation cell^{-1} (Table I).

Measurements Expressed on a Leaf Area Basis

The mean rates of photosynthetic carbon uptake per unit leaf area were lowest in the diploid, highest in the decaploid, and similar in 4×, 6×, and 8× (Fig. 4A). The lower mean rate in the diploid plants was not statistically different from the values for the 4×, 6×, and 8× plants, due to the higher standard deviation in the diploid. The correlation coefficient for photosynthesis per leaf area versus ploidal level was $r = 0.866$ (not shown), largely due to the higher rate in the 10× plants.

The amount of Chl per unit leaf area was similar at all ploidal levels, but 2× plants had lower levels than 10× plants (Fig. 4B). DNA per cm^2 leaf was lowest at 2× and highest at 10× and was intermediate at the other three ploidal levels (Fig. 4C). This was similar to the pattern of photosynthesis; therefore, the rate of photosynthesis per leaf area was highly correlated with the amount of DNA per leaf area (Fig. 4D).

In analyzing the number of mesophyll cells per leaf area there was a large variation among the three replicates in the 2× and 4× plants. However, considering the averages, the number of mesophyll and bundle sheath cells per unit leaf area decreased from the 2× to 6× level, with no change between the 6× and 10× levels (Fig. 5). Considering the change in cell volume (Fig. 3) and cell number per leaf area, there was a negative, but insignificant ($P > 0.05$) correlation between number of cells per leaf area and volumes of mesophyll and bundle sheath cells (Table I).

Measurements Expressed on a DNA and Chl Basis

As noted above, the amounts of DNA per cell and Chl per cell, as well as photosynthesis rate per cell, were highly correlated with ploidal level. Therefore, the correlation was very weak ($P > 0.05$) between the rates of photosynthesis on a DNA ($r = 0.678$) or Chl ($r = 0.082$) basis versus ploidal level.

Table I. *Linear Correlations between Various Factors Affected by Polyploidy in Five Ploidal Levels of A. confertifolia*

CO_2 fixed is pmol·h^{-1}. Confidence levels (P) are 0.01 and 0.05 for correlation coefficient values (r) of 0.959 and 0.878, respectively. $n = 5$.

Factors		r	P
y	*x*		
pg DNA·cell^{-1}	CO_2 fixed·cell^{-1}	0.949	<0.05
Enzyme activities (pmol·cell^{-1}·h^{-1})			
RuBPC	CO_2 fixed·cell^{-1}	0.892	<0.05
NAD-ME	CO_2 fixed·cell^{-1}	0.856	>0.05
PEPC	CO_2 fixed·cell^{-1}	0.843	>0.05
RuBPC	μm^3 cell volume	0.922	<0.05
NAD-ME	μm^3 cell volume	0.870	>0.05
PEPC	μm^3 cell volume	0.676	>0.05
(μmol·mg DNA^{-1}·h^{-1})			
RuBPC	CO_2 fixed·mg DNA^{-1}	0.882	<0.05
NAD-ME	CO_2 fixed·mg DNA^{-1}	0.911	<0.05
PEPC	CO_2 fixed·mg DNA^{-1}	0.715	>0.05
mg PEPC·mg DNA^{-1}	CO_2 fixed·mg DNA^{-1}	0.709	>0.05
μm^3 cell volume			
Bundle sheath	CO_2 fixed·cell^{-1}	0.901	<0.05
Mesophyll	CO_2 fixed·cell^{-1}	0.913	<0.05
No. cells·cm^{-2} leaf			
Bundle sheath	μm^3 cell volume	−0.681	>0.05
Mesophyll	μm^3 cell volume	−0.821	>0.05

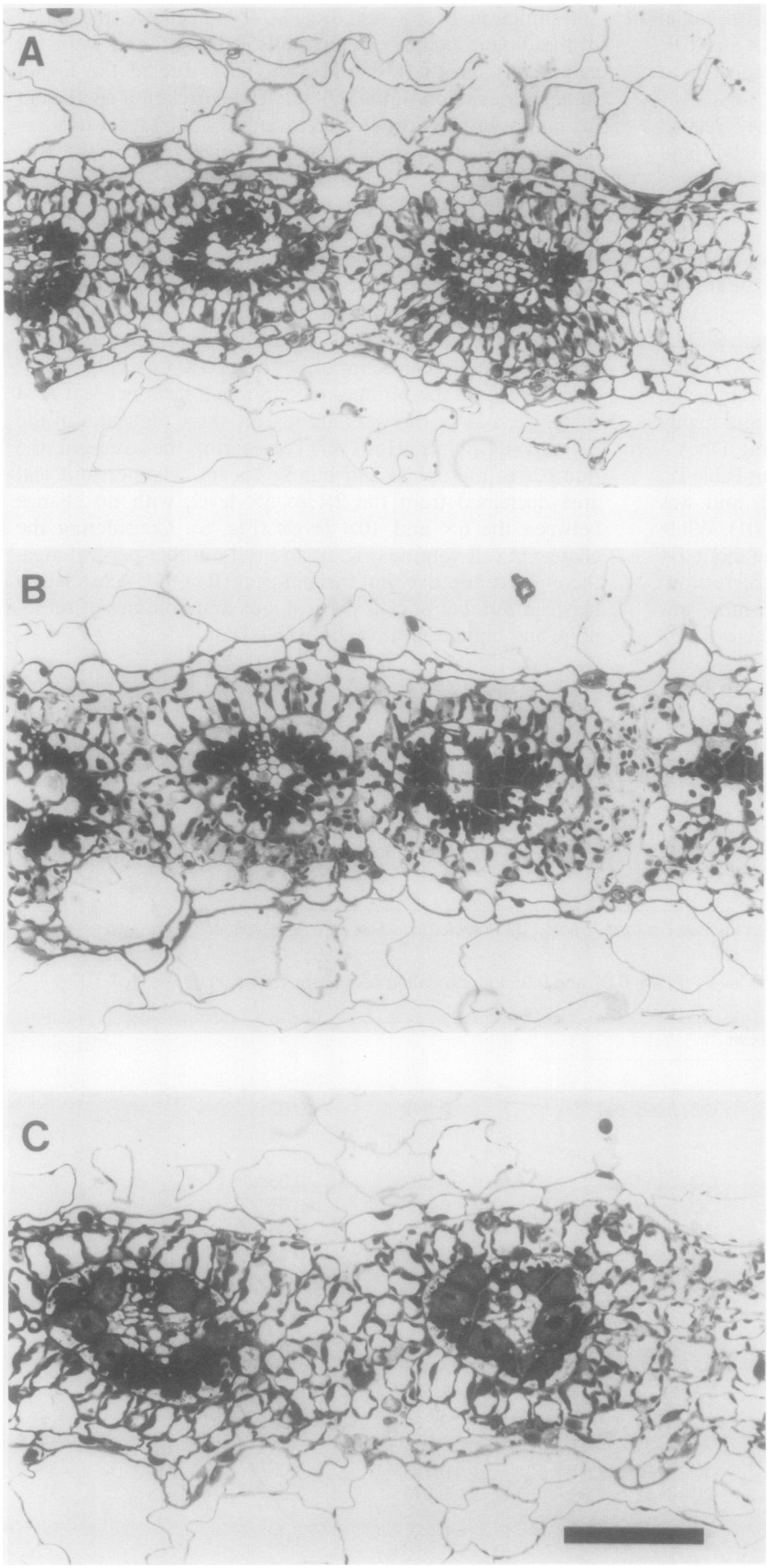

Figure 2. Light micrographs of leaf cross sections. A, 2×; B, 4×; C, 10×. Bar = 80 μm.

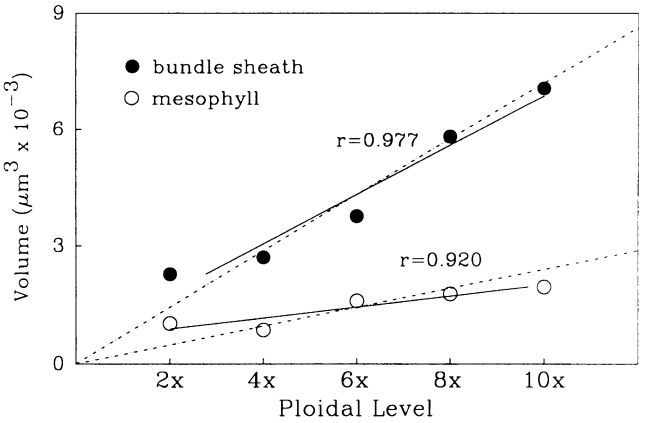

Figure 3. Volumes of protoplasts in enzymatic digestions of leaves filtered through 211 μm and 80 μm nylon nets. Diameters of protoplasts were measured with an ocular micrometer on a light microscope with a 40× objective. *Solid lines* are regression analyses. *Broken lines* are drawn from the origin through the regression lines at 6× (representing a theoretical doubling of function with doubling of ploidy).

Similarly, enzymes activities per unit Chl were not correlated with ploidal level (data not shown).

The rates of photosynthesis per unit DNA, and activities of the three photosynthetic enzymes per unit DNA were not correlated with ploidal level, although photosynthesis and enzyme activities showed a similar pattern of change with change in ploidy. The rates were highest in the 2× plants, which suggests a slightly less than proportional gene dosage effect on gene products (data not shown).

DISCUSSION

Photosynthetic rates per unit leaf area are dependent on the photosynthetic rate per cell times the number of cells per unit leaf area. In C_4 plants, the photosynthetic capacity of the cells is determined by the activities of enzymes in the carbon-fixation pathway if light and CO_2 are not limiting (4,20). These activities, in turn, are influenced by gene dosage and cellular volume in polyploid plants. The number of cells per unit leaf area is determined by the volume of cells and by leaf structure. Larger cells in polyploid plants have higher photosynthetic capacity than smaller cells with lower chromosome numbers (10,22,23). Therefore, if the numbers of photosynthetic cells per unit leaf area in polyploid plants decline in proportion to the increase in cell volume, then photosynthetic rates per unit leaf area (and per unit DNA) may be the same as in plants having smaller cells but with more cells per unit leaf area. This was recently demonstrated in the autotetraploid C_4 species *Pennisetum americanum* which arose spontaneously in a genetically inbred line (23). However, in naturally occurring populations of tetraploid and octaploid plants of the C_4 species *Panicum virgatum*, photosynthesis rates per cell were doubled in the octaploid, but mesophyll and bundle sheath cell volumes only increased by 15 to 20%, which resulted in the octaploid having a higher photosynthetic rate per leaf area (22). In the present study, a more extensive investigation of polyploidy was made with the C_4 dicot species *Atriplex confertifolia* by using natural populations having a range in ploidy from 2× to 10×.

In the polyploid complex of *A. confertifolia*, there was evidence that suggested a near proportional effect of gene dosage on certain gene products (based on DNA content, activity of some enzymes and photosynthesis per cell) with doubling of ploidy. Photosynthetic rates per cell were highly correlated with ploidal level and were correlated ($P < 0.05$) with average DNA content per cell and cell volume, as well as with the activity of RuBPC per bundle sheath cell. Activities of RuBPC and NAD-ME per bundle sheath cell were correlated with bundle sheath cell volume at 95 and 90% confidence levels, respectively. Thus, photosynthetic capacity per cell increased with ploidal level in proportion to increases in cell volume, content of DNA, and activities of photosynthetic enzymes. The rate of photosynthesis per leaf area was strongly correlated with DNA per leaf area (Fig. 4D). The amount of DNA per unit leaf area is a function of the number of cells per unit leaf area as discussed below and the DNA content per cell.

In this polyploid complex there were differences at the lower versus higher ploidy levels brought about by the interaction of changes in cell volumes and numbers per unit leaf area. At the lower ploidal levels, the number of cells per unit leaf area decreased as cell volume increased. At higher ploidal levels, cell volume continued to increase, but cell numbers per area remained constant, indicating a change in cell packing at higher ploidal levels (leaves were not thicker at higher ploidal levels) (Fig. 2). At the highest ploidal level, photosynthesis per unit leaf area increased (68% higher in 10× compared to 2×, Fig. 4A), because the increased cellular capacity for CO_2 fixation was not offset by a decrease in the number of bundle sheath cells per unit leaf area.

Interestingly, bundle sheath cells were more affected than mesophyll cells by changes in ploidy. Bundle sheath cell volumes increased proportionally more than mesophyll cell volumes. Chl per bundle sheath cell increased markedly, while Chl per mesophyll cell stayed essentially constant. The activity of RuBPC per bundle sheath cell was correlated with ploidal level and with photosynthesis per cell. Bundle sheath enzyme activities per unit DNA were correlated with photosynthesis per unit DNA at a 95% confidence level (Table I). There was no apparent gene dosage effect on the amount of PEPC protein per cell, and activities and amounts of PEPC were not significantly correlated (at the 95% level) with photosynthetic rates expressed per cell or per unit DNA. Thus, changes in bundle sheath cells were more apparent and may have more influence on the relative photosynthetic rates in polyploid *A. confertifolia* than those in mesophyll cells. Usuda *et al.* (20) also found that in 10 C_4 species photosynthesis per unit leaf area was correlated more with activity of RuBPC than with PEPC on an area basis.

In summary, as chromosome number increased in polyploid races of *A. confertifolia*, DNA per cell, enzyme activity per cell, cell volume, and photosynthesis per cell all increased. The number of cells per unit leaf area decreased only at lower ploidal levels from 2× up to 6×. Thus, photosynthesis on a leaf area basis was similar in 2×, 4×, and 6× plants (mean somewhat lower in 2×), because increased photosynthetic

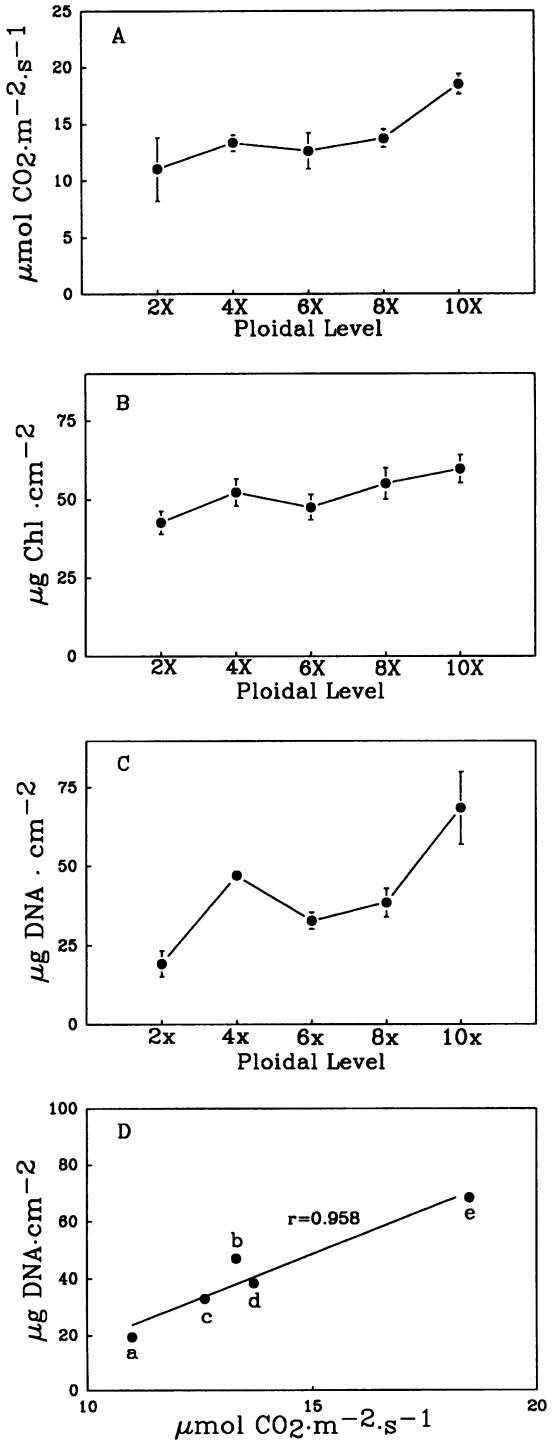

Figure 4. A, Photosynthetic carbon uptake per unit leaf measured by infrared gas analysis. The photosynthetic rate of 6× plants from the growth chamber was 7.34 ± 0.86 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. *Bars* indicate standard deviations. B and C, Contents of Chl and DNA per unit leaf area. DNA was measured by fluorescent spectroscopy in extracts of leaves used to measure photosynthetic gas exchange. Chl was measured in 95% ethanol extracts of leaf discs. D, Linear regression of DNA content per leaf area *versus* photosynthetic rate per leaf area in different ploidal levels of *A. confertifolia*. a, 2×; b, 4×; c, 6×; d, 8×; e, 10×.

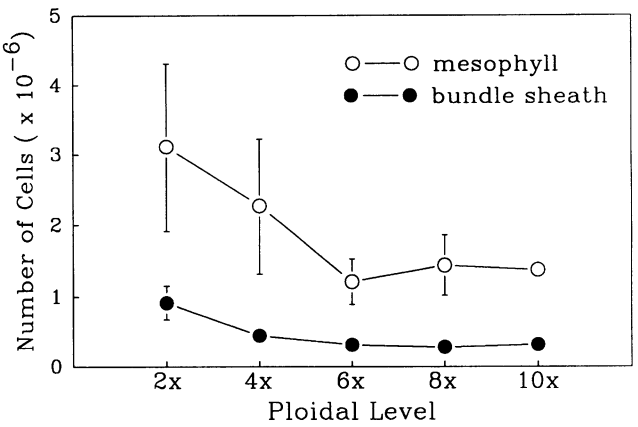

Figure 5. Number of mesophyll and bundle sheath cells per unit leaf area in chromium trioxide digestions of leaf discs. Cells were counted on a 0.2 mm depth hemocytometer with a Fuchs-Rosenthal pattern. Cells were counted in the four corner squares and one central 1 mm^2 square of the pattern. Three replicate discs were digested at each ploidal level. *Bars* indicate standard deviations.

capacity per cell tended to be offset by a decreased number of cells per unit leaf area. Photosynthetic capacity per cell was highest in 10×, while the number of cells per leaf area was the same in 10× and 8×. Thus, the photosynthesis per unit leaf area was highest in 10×. For maximum photosynthetic rates on a leaf area basis, the decaploid plants appear to have the optimum combination of chromosome number, cell volume, and number of cells per unit leaf area. Decaploids of *A. confertifolia* are described as being the most robust (largest, implying more competitive) and highest ploidal level of this species in nature (18).

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