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*Chrysothamnus nauseosus* ssp. *albicaulis*

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CHLOROPLAST ULTRASTRUCTURE IN THE DESERT SHRUB
CHRYSOATHAMNUS NAUSEOSUS SSP. ALBICAULUS

Craig E. Coleman¹ and William R. Andersen¹

ABSTRACT.—Ultrastructure of the chloroplasts of white rubber rabbitbrush (Chrysothamnus nauseosus (Pallas) Britt. ssp. albicaulis) was observed with electron microscopy. In addition, leaf anatomy was observed with light microscopy. Previously, it had been reported that the leaves of this desert shrub exhibited a relatively high rate of photosynthesis when compared to other C₃ plants. Comparisons with chloroplasts of other C₃ and C₄ plants demonstrated a reduced amount of granal stacking in the rabbitbrush. However, the classification of rabbitbrush as a C₄ plant is confirmed. RUBP-carboxylase concentration is reported at about 450 mg:ml¹ stromal space based on the estimation of 1 mg of chlorophyll per 25 ul of stromal space in a normal C₃ chloroplast and data from an assay to determine the ratio of RUBP-carboxylase to chlorophyll.

Recent interest in the desert shrub, Chrysothamnus nauseosus (Pallas) Britt. ssp. albicaulis (white rubber rabbitbrush), has led to a number of studies related to its ecology and physiology (McArthur et. al. 1979). Of particular interest has been its study as a non-traditional source of rubber. Ostler (1980) reported rubber acquisitions as high as 6% rubber per unit dry weight from the plant. As a result of this finding, studies are being conducted to discover some of the factors controlling the production of rubber. Recently work was done to determine some basic aspects of the photosynthetic characteristics of the plant. It was discovered that, under non-stressed conditions, white rubber rabbitbrush exhibits a relatively high rate of photosynthesis when compared to other C₃ plants (Davis et. al., 1985), and it was felt that an electron microscopic analysis of the chloroplast ultrastructure might shed some additional light on the problem. This paper, therefore, presents the result of this analysis, along with anatomical data obtained from light microscopy. Additionally, we report the approximate RUBP-carboxylase concentration in the stromal space based on an estimate for the concentration of chlorophyll in the stromal space.

MATERIALS AND METHODS

Leaves were removed from young branches of white rubber rabbitbrush plants growing in the greenhouse at Brigham Young University. The leaves were cut into sections approximately 1–2 mm long and placed immediately in 0.2 M sodium cacodylate buffered (pH 7.3) 2% glutaraldehyde 3% acrolein (v/v) solution for two hours for fixation. After being washed for one hour with a 1:1 solution of buffer and distilled water, the material was stained with 2% osmium tetroxide (w/v) diluted 1:1 with the sodium cacodylate buffer. The material was again washed with the buffer solution for one hour and subsequently dehydrated using an ethyl alcohol series.

The material was then embedded in Spurr’s resin (Spurr 1969) by first rinsing three times in 100% acetone. It was allowed to stand for one hour each in first a 25% resin to acetone solution (v/v), then a 75% solution before finally embedding in 100% resin. Sections were obtained using a glass knife in a Porter-Blum MT-2 ultra-microtome. For electron microscopy, sections were mounted on copper grids previously coated with formvar and a light layer of carbon. Lead citrate was used as a poststain as previously described (Reynolds 1963). The sections were observed and photographed with a Phillips EM 400 transmission electron microscope. For light microscopy, sections were taken from the same resin-embedded material as used for electron microscopy. These sections were mounted on a glass slide and poststained with a 1% Toluidine Blue, 1% Azure II, and 1% NaHCO₃.

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stain. An enzyme assay to determine the ratio of RUBP-carboxylase to chlorophyll was performed as described previously (Davis et al. 1985).

**Results**

**Light Microscopy.**—Transverse sections were viewed with the light microscope (Fig. 1) with the intent to compare the anatomy of the white rubber rabbitbrush with that of known C₃ and C₄ plants. Chloroplasts are located exclusively in the mesophyll cells and are lacking in the bundle sheath cells. This indicates the presence of C₃ metabolism (Laetsch 1974).

**Electron Microscopy.**—Typical chloroplasts observed with the electron microscope are shown in Figures 2 and 3. It should be noted that in all the chloroplasts observed there existed a uniformity of structure, in other words, a complete lack of dimorphism, which is usually exhibited in chloroplasts of C₄ plants (Laetsch 1968). Furthermore, chloroplast size and distribution do not seem to vary from one section of the leaf tissue to another.

The enzyme assay to determine the ratio of RUBP-carboxylase to chlorophyll in the chloroplast yielded a result of $11.24 \pm 0.81$ mg RUBP-carboxylase.

**Discussion**

To determine if the chloroplasts of the white rubber rabbitbrush were unusual in any way, their ultrastructure was compared to the ultrastructure of the chloroplasts from several C₃ and C₄ plants reported in the literature. Thus, comparisons were made with chloroplasts from radish (Rufner et al. 1984), barley (Robertson and Laetsch 1974), rye (Huner 1984), tobacco (Laetsch and Stetler 1965, Kasperbauer and Hamilton 1984), bean (Weier and Thomson 1962), rice (Miyake and Maeda 1976), spinach, and tomato (Rufner and Barker 1984). Although typical of C₃ anatomy, we note that the grana in the chloroplasts of the white rubber rabbitbrush seem to be less stacked in comparison to chloroplasts of mesophyll cells in both C₃ and C₄ plants. This, however, could be due to a variety of
factors, including the age of the leaf (Robertson and Laetsch 1974), the amount of light (Apel 1983), and the quality of light (Kasperbauer and Hamilton 1984) being absorbed by the leaves of the plants, or the absence of vital nutrients and mineral in the soil, such as iron (Rufner and Barker 1984). A most likely explanation for the reduced stacking is the age of the chloroplasts, since the leaves, although fully expanded, were taken from young shoots.

Heldt (1979) estimated the chlorophyll content in a normal C₃ chloroplast to be about 1 mg per 25 ul of stromal space. Data from an enzyme assay reported previously indicate the ratio of RUBP-carboxylase to chlorophyll in rabbitbrush to be 12.93 ± 0.74 mg RUBP-carboxylase · mg⁻¹ chlorophyll (Davis et. al. 1985). This assay was repeated for the purpose of this paper and, as already stated, yielded a value of 11.24 ± 0.81 mg · mg⁻¹. Assuming Heldt’s estimation of stromal space to be reasonable in this case, the concentration of RUBP-carboxylase in rabbitbrush chloroplasts is about 450 mg · ml⁻¹. This is a high concentration of the enzyme as compared to several other C₃ plants (values, on the average, are reported around 200–250 mg · ml⁻¹) (Ashton 1982, Kawashima and Mitake 1969; Lyttleton and Ts’o 1958; Molin et. al. 1982) and may be responsible for the increased rate of photosynthesis as observed in the plant.

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LITERATURE CITED


Fig. 3. Transmission electron micrograph of a rabbitbrush chloroplast with a prominent starch grain showing cell wall (cw), starch grain (sg)(X45000).


GENETIC VARIATION OF WOODRATS (NEOTOMA CINEREA) AND DEER MICE (PEROMYSCUS MANICULATUS) ON MONTANE HABITAT ISLANDS IN THE GREAT BASIN

William T. Mewaldt1,2 and Stephen H. Jenkins1

ABSTRACT.—Seventeen loci were examined for polymorphism in four populations of Neotoma cinerea and Peromyscus maniculatus on isolated mountain ranges in the Great Basin, one population of each in the Sierra Nevada, and one of each in the Rocky Mountains. All Peromyscus populations had higher levels of heterozygosity than syntopic Neotoma populations. Results indicate; interstriae 1 moderately elevated, very slightly higher than interstriae 3, with a median

Populations of species restricted to terrestrial habitat islands may be similar to those on oceanic islands in patterns of gene frequency change over time (Kilpatrick 1981). For example, Glover et al. (1977) studied pikas (Ochotona princeps), which are generally restricted to talus slopes and other rocky habitats at high elevations. Their findings of low heterozygosity within populations are consistent with patterns on oceanic islands reviewed by Soule (1976) and Kilpatrick (1981), who concluded that the stochastic processes of founder effect and genetic drift may cause unpredictable shifts in gene frequency and reduced heterozygosity. The distribution of montane habitats in the Great Basin of the western U.S. provides an opportunity for “island-mainland” comparisons of montane mammals. In the Great Basin, isolated patches of forested habitat act as refugia for many populations of mammals that apparently cannot live in or cross the intervening deserts (Brown 1971). As recently as 8,000 years ago, climatic conditions were such that forests (interspersed with pluvial lakes) existed continuously across the Great Basin from the Sierra Nevada to the Rocky Mountains. Since that time, the climate has become drier, resulting in isolation of montane habitat at higher elevations of the mountain ranges (Wells 1983). We analyzed allozymes in several populations of two cricetid rodent species, Neotoma cinerea acraia (Elliot) (bushy tailed woodrat) and Peromyscus maniculatus sonoriensis (LeConte) (deer mouse), to test some genetic predictions of the hypothesis that stochastic effects should be more pronounced for isolated island populations than for “mainland” populations. Peromyscus maniculatus sonoriensis is distributed continuously across the Great Basin (Hall 1946), whereas Neotoma cinerea acraia is found in isolated populations on most of the Basin ranges (Brown 1971).

Biochemical variation within at least 20 species of Peromyscus has been reported (e.g., Sellander et al. 1971, Kilpatrick and Zimmerman 1976, Zimmerman et al. 1978, Avise et al. 1979, Gill 1980). Of special interest here are the studies by Avise et al. (1979) and Gill (1980), both of which included P. m. sonoriensis. Avise et al. reported mean heterozygosity (H) values for populations of this subspecies ranging from 0.074 to 0.124, whereas Gill found an H of 0.118.

Electrophoretic studies of Neotoma are few. Mascarelllo (1978) studied three chromosomal races of Neotoma lepida in the southwest but did not report H values, whereas Zimmerman and Nejtek (1977) reported H values for three semispecies of Neotoma (N. albigula, N. micropus, and N. floridana) in southern North America ranging from 0.024 to 0.140, with an average of 0.078. Heterozygosity measures for populations of N. cinerea have not been reported.

MATERIALS AND METHODS

Six mountain ranges with populations of N. c. acraia and P. m. sonoriensis were chosen

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