tween phytoplankton nitrogen demand and de novo input through nitrogen fixation in a supposedly nitrogen-limited system (*24, 27*), it is enigmatic that diazotrophic phytoplankton (that is, nitrogen-fixing cyanobacteria) are not more prevalent. In whole-lake experiments, Schindler (*28*) reported that nitrogen limitation (low nitrogen-to-phosphorus ratio) regularly results in nuisance blooms of nitrogen-fixing cyanobacteria, whereas phosphorus limitation (high nitrogen-to-phosphorus ratio) yields phytoplankton populations dominated by eukaryotic algae (*28*). He also found that, for nitrogen-limited lakes, nitrogen fixation contributed substantially (19 to 38 percent) to the total nitrogen economy. Further circumstantial evidence indicative of a nonnutrient limited growth status for oceanic phytoplankton is found in the work of Goldman *et al.* (*26*), who suggest that phytoplankton in the marine environment are growing at near maximum rates, despite apparent nutrient impoverishment. In the sea, diazotrophic bacteria (including the cyanobacteria) may themselves be limited by either availability of other trace nutrient requirements (for example, molybdenum, iron, or phosphorus) reducing power, or oxygen inactivation (*29*). An alternative explanation is that growth or productivity of phytoplankton, or both, is in general not nitrogen-limited but is controlled instead by some other biological (for example, grazing), chemical (macro- or micronutrient), or physical (for example, light or temperature) factor.

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30. Previous research has shown virtually all N_2 fixation by *Oscillatoria* occurs in the upper 50 m of the water column (*3–7*). Field and laboratory investigations have indicated very little photosynthesis by *Oscillatoria* in water colder than 20°C. [R. Marumo and S. Nagasawa, *Bull. Plankton Soc. Jpn.* **23**, 19 (1976)]; E. Wada, A. Hattori, T. Miyasaki, in *Studies on the Community of Pelagic Blue-Green Algae, 1972–1974*, R. Marumo, Ed. (Ocean Research Institute, University of Tokyo. 1976).
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Late Quaternary Zonation of Vegetation in the Eastern Grand Canyon

Abstract. *Fossil assemblages from 53 packrat middens indicate which plant species were dominant during the last 24,000 years in the eastern Grand Canyon. Past vegetational patterns show associations that cannot be attributed to simple elevational displacement of the modern zones. A model emphasizing a latitudinal shift of climatic values is proposed.*

In an early life zone study of the San Francisco Peaks and adjacent parts of the Grand Canyon in northern Arizona, Merriam (*1*) concluded that modern vegetation is distributed along topographic gradients in elevational zones that resemble latitudinal zones. The similarity of elevational and latitudinal zonation of vegetation in the western United States has made the interpretation of past climates through the fossil record difficult. Patterns of climate in elevational zones may be different from those in latitudinal zones, but paleoecological records often lack the detail necessary to distinguish between elevational and latitudinal analogs of vegetation and hence the nature of past climate.

Analysis of fossil plant remains from packrat (*Neotoma* spp.) middens (*2–4*), unlike pollen analysis, often allows specific identification in difficult genera such as *Pinus* and *Quercus*. The local nature of fossil assemblages from middens—material from within 100 m of the site (*4*)—allows reconstruction of vegetational distributions along the elevational gradient as well as along the moisture gradient from insolated (xeric) to shaded (mesic) slopes. Radiocarbon dates for 48 of 53 packrat middens collected along an 1800-m elevational gradient in the eastern Grand Canyon (*5*) ranged from 34,300 to 1170 years old. The present vegetation, as described from 131 vegetational relevés (*6*) or plots, was compared with the fossil assemblages.

The elevational zonation of vegetation

throughout the last 24,000 years in the eastern Grand Canyon (Fig. 1) indicates that during the full-glacial period (21,000 to 15,000 years ago) (*7*) most plant species occurred 600 to 1000 m lower than they do today on sites similar in aspect. Although the shift toward modern vegetational zonation began in the Late Wisconsin (15,000 to 11,000 years ago), some woodland and fir forest species remained well below their modern limits into the early Holocene (11,000 to 8500 years ago) (*8*). The loss of extralocal species (those not present today) was apparently not simultaneous at all sites, suggesting that a gradual transition from Pleistocene extralocal species to modern dominants occurred between 13,500 and 8500 years ago. These results, together with those from a record of 47 packrat middens collected at lower elevations in the western Grand Canyon (*9*), support the concept of a time-transgressive boundary between the Pleistocene and Holocene (*10*). Significant changes continued during the Holocene. Mid-Holocene middens from the eastern Grand Canyon suggest an upward displacement of pinyon-juniper woodland between 8500 and 6800 years ago. These records of late Quaternary changes in vegetation are consistent with most chronologies from the southwest (*11*).

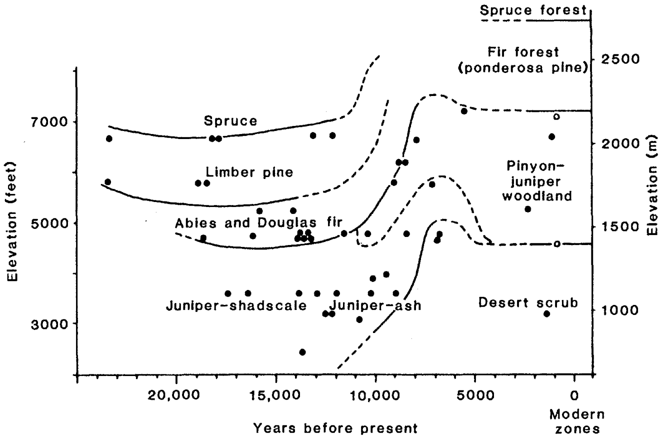

Fig. 1. The zonation of dominant taxa during the past 24,000 years in the eastern Grand Canyon. Lines represent the lower elevational limits of taxa inferred for western-facing, moderately protected slopes. Dashed lines are suggested limits. Data points represent ^{14}C-dated (●) or archeologically dated (○) plant assemblages.

Insight into past environments may be gained by investigating how vegetation zones have changed over the past 21,000 years. Pleistocene zones were not simply depressed versions of modern zones; the composition of the plant associations has changed through time. In order to describe these changes, fossil localities were plotted according to elevation and insolation (Fig. 2) (*12*) to show the distributions of dominant species during three time periods in the Grand Canyon (Table 1).

On the basis of similarity to corresponding modern relevés (*5*), late Holocene middens appear to represent accurately the dominant species within 30 m of the fossil site. Six middens less than 1200 years old averaged 77 percent similarity (*5*) by Sørensen's index (*6*), but ten middens from the full-glacial time averaged only 19 percent similarity compared with their corresponding modern relevés. Because plant remains of most species in the late Holocene middens reach their highest concentrations at sites where the percentage of ground cover is greatest, it is reasonable to assume that macrofossil concentration is a rough indicator of past importance of dominant species.

Table 1. Radiocarbon dates on material from selected middens from the eastern Grand Canyon during three time periods.

Number (Fig. 2)	Date (years)	Laboratory number	Material dated	Deposit elevation (m)	Slope aspect
			Full glacial		
1	17,950 ± 600	RL-1180	*Pinus flexilis*	2020	W
2	18,130 ± 350	A-1964	*Pinus flexilis*	2020	W
3	18,800 ± 800	RL-1178	*Neotoma* pellets	1770	SW
4	18,490 ± 660	A-2023	*Neotoma* pellets	1770	SW
5	15,840 ± 310	WK-176	*Pseudotsuga menziesii*	1600	NE
6	16,165 ± 615	GX-6302	*Pseudotsuga menziesii*	1450	NW
7	18,630 ± 310	A-1798	*Juniperus* sp.	1450	W
8	20,630 ± 470	A-2337	*Juniperus* sp.	1450	SW
9	17,400 ± 450	WK-179	*Juniperus* sp.	1100	SE
10	16,400 ± 190	WK-165	*Juniperus* sp.	1100	SE
			Early Holocene		
11	8900 ± 340	WK-150	*Juniperus* sp.	1900	SW
12	8590 ± 110	WK-147	*Juniperus* sp.	1900	SW
	8430 ± 400	WK-149	*Pseudotsuga menziesii*		
13	9070 ± 350	A-2024	*Neotoma* pellets	1770	NE
14	10,290 ± 150	A-1808	*Juniperus* sp.	1460	W
15	9400 ± 270	WK-177	*Juniperus* sp.	1220	SSW
16	10,110 ± 100	A-1779	*Juniperus* sp.	1200	W
17	10,150 ± 120	WK-146	*Juniperus* sp.	1100	W
18	8957 ± 96	A-1765	*Juniperus* sp.	1100	W
19	10,760 ± 260	WK-150	*Juniperus* sp.	950	N
			Late Holocene		
20	1220 ± 70	A-2336	*Pinus edulis*	2070	NE
21	1170 ± 80	A-1995	*Pinus edulis*	2050	SW
22	<1000		*Zea mays*	2170	SE
23	<1000		*Zea mays*	1450	W
24	1345 ± 135	GX-6304	*Neotoma* pellets	970	SW
25	<100		Green leafy material	1100	W
26	<100		Green leafy material	1100	W

The fossil record from the ten full-glacial middens suggests plant assemblages which are common today in the northeastern Great Basin and northern Utah (*13*). Low-elevation xeric slopes are dominated by Utah juniper (*Juniperus osteosperma*) and shadscale (*Atriplex confertifolia*), with sagebrush (*Artemisia* cf. *tridentata*) concentrated on the lower mesic slopes. High-elevation xeric slopes are dominated by limber pine (*Pinus flexilis*), with Douglas fir (*Pseudotsuga menziesii*) and white fir (*Abies concolor*) dominating the high mesic slopes. Spruce (*Picea* sp.) is present at elevations above 2000 m. Furthermore, these assemblages lack many Grand Canyon dominants which also do not occur in northern Utah today, such as pinyon pine (*Pinus edulis*), ponderosa pine (*Pinus ponderosa*), cliffrose (*Cowania mexicana*), manzanita (*Arctostaphylos pungens*), and shrub live oak (*Quercus turbinella*). Some species are represented in the full-glacial assemblages from below 1500 m that are not now present in northern Utah (for example, *Agave utahensis* and *Rosa* cf. *stellata*), but the absence of low-elevation

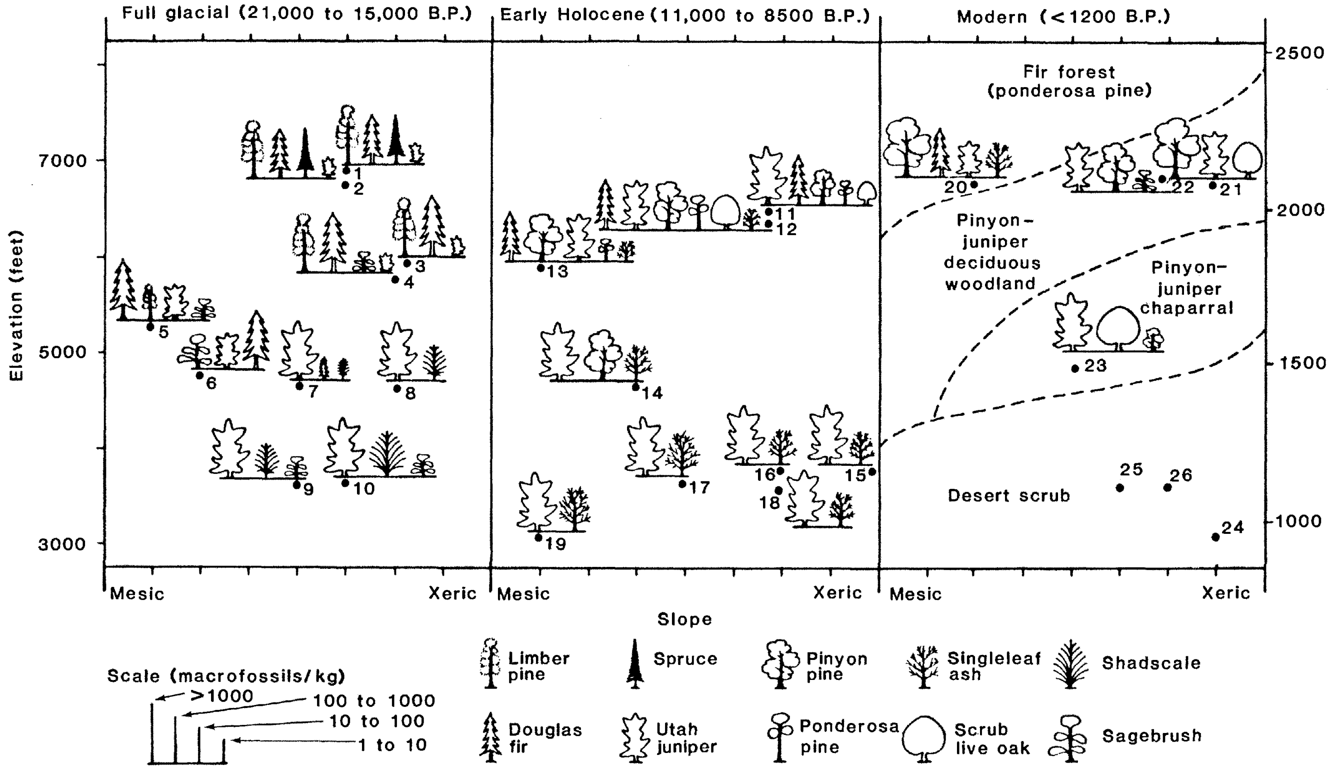

Fig. 2. The major associates of middens during three selected time periods. Sizes of symbols are varied to illustrate fossil concentrations, as shown on the scale. Dots represent the location of fossil assemblages with respect to the elevation and insolation of the sites, and numbers refer to radiocarbon dates (Table 1).

habitats in northern Utah (*14*) prevents a full comparison.

The similarities in vegetational associations between the full-glacial Grand Canyon and modern northern Utah suggest that a latitudinal shift in climatic values provides a better model for inferring the nature of full-glacial climates than does an elevational depression and that the full-glacial climate of the Grand Canyon was colder in all seasons than it is today, had a wider range between summer means and winter means (less temperate), and had a mean annual precipitation approximating modern values. Unlike the present biseasonal regime (*15*), the precipitation fell predominantly in winter.

A winter-dominated precipitation regime is consistent with other studies of the full-glacial southwest, although the implications of this cold-dry model are inconsistent with the moist-equable climates visualized in the low-elevation deserts at this time (*4, 5*). Other studies in the southwest, but at higher latitudes (*16*), also portray a full-glacial climate with approximately modern precipitation values. As is the case today, a major climatic boundary must have been present between the low-elevation deserts and the higher elevation areas in the Great Basin and the Colorado Plateau. Latitudinal displacement of climates is probably a poor model in the low-elevation deserts because many geographic variables change at 36° latitude (*17*).

Eight early Holocene middens record associations very different from those of the full glacial. Douglas fir, white fir, and Utah juniper are recorded at least 350 m below their modern distributions. For the first time in this fossil series pinyon pine, ponderosa pine, cliffrose, shrub live oak, New Mexico locust (*Robinea neomexicana*), and netleaf hackberry (*Celtis reticulata*) are recorded. Singleleaf ash (*Fraxinus anomala*) and hop tree (*Ptelea trifoliata* var. *pallida*) are represented on slopes more xeric than those they occupy today. Similar associations are found today along the Mogollon Rim 200 km south of the study area. These observations suggest an early Holocene increase in summer monsoon precipitation and support simulated climatic circulation models of 9000 years ago (*18*) and postulated late Wisconsin–early Holocene maximum pluvial lake levels (*19, 20*).

Individual plant species have responded differently to climatic changes. In the full-glacial period, Utah juniper, Douglas fir, white fir, and spruce are recorded 600 to 800 m lower than they occur today. Limber pine was present at least 1000 m below its modern limit. Shadscale was simply more abundant within its modern range, and pinyon pine was not recorded in the eastern Grand Canyon. These changes produced associations with no modern counterparts at any elevation in the study area. The fossil associations are most similar to modern associations 450 km north of the study area, supporting a model of latitudinal displacement of climate.

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High Efficiency Latency and Activation of Herpes Simplex Virus in Human Cells

Abstract. *Herpes simplex virus (HSV) exists in humans in a latent form that can be activated. To characterize the molecular basis of the cell-virus interactions and to analyze the state of the latent HSV genome, an in vitro model system was established. In this system a large fraction of the latently infected cells contain an HSV genome that can be activated. Cell survival was reduced minimally after repression of high multiplicity HSV type 1 (HSV-1) infection of human fibroblast cells with (E)-5-(2-bromovinyl)-2′-deoxyuridine in combination with human leukocyte interferon (IFN-α). A minimum of 1 to 3 percent of the surviving cells contained an HSV genome that could be activated either by human cytomegalovirus superinfection or reduction in incubation temperature.*

Herpes simplex virus (HSV) can be harbored in animals and humans without signs of clinical disease (*1*). The establishment and maintenance of the latent state, the latent form of the HSV genome, and the mechanism of reactivation are foci of current research. In addition to in vivo studies of HSV latency (*1*), in vitro model systems have made it possible to study the maintenance and expression of virus genetic information in latent HSV infections (*2, 3*). We previously described infection of human embryo lung (HEL) fibroblast cells with either HSV type 1 (HSV-1) or type 2 (HSV-2) wherein virus replication was blocked by treatment of infected cultures for 7 days with arabinosyl cytosine (ara-C); (*4–6*). Infectious virus was maintained in a repressed form by elevating the incubation temperature after removal of ara-C from 37° to 39.5°C for HSV-2 (*7*) or to 40.5°C for HSV-1 (*8*). Replication of HSV-1 or HSV-2 was activated by reducing incubation temperature, or by superinfecting with human cytomegalovirus (HCMV) (*5, 6, 8*) or HSV temperature-sensitive mutants (*9*).

Repressed HSV infections were established by infection of HEL cells with low levels [0.01 to 0.2 plaque-forming unit (PFU) per cell] of virus followed by treatment with ara-C and an increase in temperature. Assays of infectious centers were performed on ara-C–treated, HSV-2–infected cells at several times after removal of inhibitor (6 to 15 days); these assays indicated that less than 0.0002 to 0.02 percent of the cells contained a virus genome that could be activated.

To characterize the state of the virus genome and the virus activation process, it was essential to increase the number of surviving cells containing a virus genome that could be activated. Inhibitors of DNA synthesis interfere with expression of late (γ) genes of HSV and at least a subset of HSV early (β) genes, while presumably allowing complete expression of the immediate early (α) genes. Reports that human interferon blocks the deleterious effects of HSV on human chromosomes (*10*) and that interferon of fibroblast origin in combination with acyclovir inhibits HSV-1 or HSV-2 replication in an additive to synergistic manner (*11, 12*), prompted the use of human leukocyte interferon (IFN-α) in combination with the relatively noncytotoxic compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) (*13*) to obtain a significant increase in surviving cells after infection with an increased multiplicity of HSV-1.

HEL cells were first treated for 24 hours with BVDU and IFN-α and infected with HSV-1 (Fig. 1). HSV-1 was not detected after the 7-day inhibitor treatment or after incubation at 40.5°C for 8 days. Infectious virus (determined by plaque assay on primary rabbit kidney cells) was not detectable in control uninfected HEL cells treated with BVDU plus IFN-α for analogous time intervals. Surviving cells obtained after BVDU plus IFN-α treatment have been maintained for at least 50 days at 40.5°C without detection of infectious virus. Virus can be activated at this time by

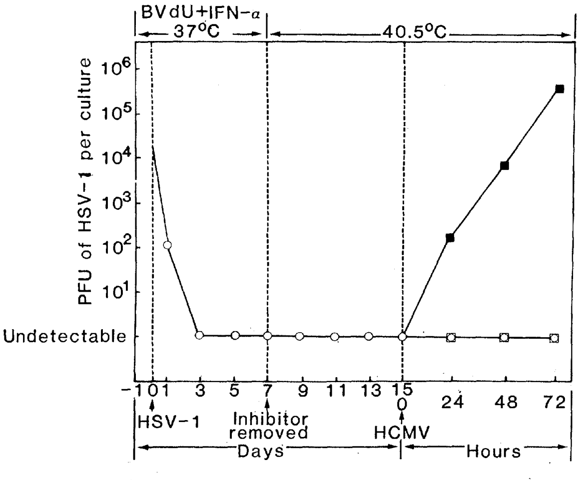

Fig. 1. Establishment and maintenance of repression interval and HCMV-mediated activation. Confluent HEL cell cultures were first treated with BVDU (10 μg/ml) and IFN-α (125 IU/ml) for 24 hours, infected with HSV-1 (2.5 PFU/cell); they were then treated at 24-hour intervals with maintenance medium containing BVDU (10 μg/ml) and IFN-α (125 IU/ml) for 7 days. The cultures were then washed twice with tris-buffered saline (*p*H 7.4); maintenance medium without inhibitors was added, and cultures were shifted from 37° to 40.5°C. Maintenance medium without inhibitors was changed every third day at the elevated temperature. Cultures were assayed for infectious virus during the treatment with BVDU plus IFN-α and after the inhibitor was removed and the temperature was increased (○). BVDU plus IFN-α-repressed, HSV-1–infected cells maintained at 40.5°C for 8 days, were superinfected with 1.0 PFU of HCMV (■) per cell or treated with mock-infecting fluid (□) prepared from uninfected (Flow 5000) cells. After adsorption, superinfected cultures were overlaid with maintenance medium at 40.5°C until the indicated harvest time and infectious virus was determined. All cultures were harvested and assayed for infectious virus by plaque assay in primary rabbit kidney cells (*5*).