

Fig. 3. Loss of photoreactivability in *E. coli* B during holding at 23°C in phosphate buffer. The remaining PR sector after liquid holding for various times is plotted on a logarithmic ordinate versus time of holding. Data derived from Fig. 2.

rapid than the control drop, suggesting that some initially photoreactivable lesions become irreversible (by either holding or PR treatment) during holding. This interpretation is further supported by the observation that after 8 hours of holding, PR treatment cannot significantly raise survival (Fig. 2), whereas the survival of cells receiving various amounts of photoreactivating light at time zero and then held for 8 hours (Fig. 1) is always higher than that obtained with holding alone. We conclude that it is likely (though admittedly on the borderline of significance) that some photoreactivable lesions become irreversible with holding of the cells in phosphate buffer.

Knowing the ultraviolet survival kinetics (virtually exponential in this region), one can calculate (3) the photoreactivable sector for any population, which is the fraction of the ultraviolet dose that appears to be eliminated by a full PR treatment. From Fig. 2 one can calculate the sector for holding plus PR treatment (using the highest experimental point on the PR curve) and subtract from it the sector that was eliminated by holding alone. This

gives the PR sector remaining after holding. For example, at 2 hours' holding,

$$\begin{aligned} \text{PR sector remaining} &= \\ \frac{\text{Dose}_{\text{UV}}(26.5\% \text{ surv.}) - \text{Dose}_{\text{UV}}(60.5\% \text{ surv.})}{\text{Dose}_{\text{UV}}(10.7\% \text{ surv.})} &= 0.36, \end{aligned}$$

which means that, after ultraviolet killing to 10.7 percent survival, and holding for 2 hours, 36 percent of the ultraviolet damage can still be photoreactivated. Figure 3 shows that the remaining photoreactivable sector decreases exponentially with time of holding, suggesting a first-order decay of photoreactivable lesions during holding. Most of this decay involves effective repair of the lesion, but a little of it seems to be a transition of the lesion into an irreversible, lethal state.

We conclude that, at these survival levels in these cells, (i) LHR treatment acts only on photoreactivable lesions, and (ii) LHR treatment may be potentially capable of acting on all the photoreactivable lesions. If the only photoreactivable lesion in these cells is the thymine dimer, then LHR treatment must act only on the thymine dimer. By definition, it does this by some "dark" reaction. That such dark reactions exist for ultraviolet damage to cells is strongly suggested by a variety of evidence (see 8). Metzger (9) has shown that a dark reaction leading to "host-cell reactivation" very probably acts on photoreactivable lesions, and it is known that holding in buffer after ultraviolet can induce a division delay upon plating (10). It may be that the action of LHR treatment is to induce a division delay that permits more time for host-cell reactivation reactions to act on the ultraviolet lesions.

We also wish to report that *E. coli* B<sub>s-1</sub> (Hill), a very ultraviolet-sensitive mutant, which is highly photoreactivable (11), showed no LHR when irradiated in the stationary phase, held in buffer, and then plated on nutrient agar. Log-phase cells, plated on nutrient agar, showed a small LHR, but only for dark survivals below 1 percent. It is of interest that this organism fails to show other dark reactivations (8), which further suggests a link between these reactivations and LHR.

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#### Pleistocene Wood Rat Middens and Climatic Change in Mohave Desert: A Record of Juniper Woodlands

**Abstract.** Leafy twigs and seeds of juniper are abundant in nine ancient *Neotoma middens* discovered in low, arid, desert ranges devoid of junipers, near Frenchman Flat, Nevada. Existing vegetation is creosote bush and other desert shrubs. Twelve radiocarbon dates suggest that the middens were deposited between 7800 to more than 40,000 years ago. Dominance of Utah juniper and absence of pinyon pine in most deposits indicates a local Pleistocene woodland climate more arid than the usual pinyon-juniper climate.

An area of more than 5000 km<sup>2</sup> in the vicinity of Frenchman Flat in southern Nevada is occupied by arid basins and ranges which are very sparsely vegetated with a low desert scrub (1) except for localized stands of bizarre Joshua trees (*Yucca brevifolia* Engelm.). The mountains immediately surrounding Frenchman Flat basin are relatively low in elevation and do not support coniferous forest or woodland. Even the relatively xerophytic woodland species of juniper and pinyon pine [*Juniperus osteosperma* (Torr.) Little and *Pinus monophylla* Torr. and Frem.] are totally lacking, although they are present on higher ranges such as the Spring Mountains and Shoshone Mountain, some 32 km from the south side of Frenchman Flat.

Therefore, a major climatic change is indicated by the fact that *Juniperus osteosperma* is one of the abundant

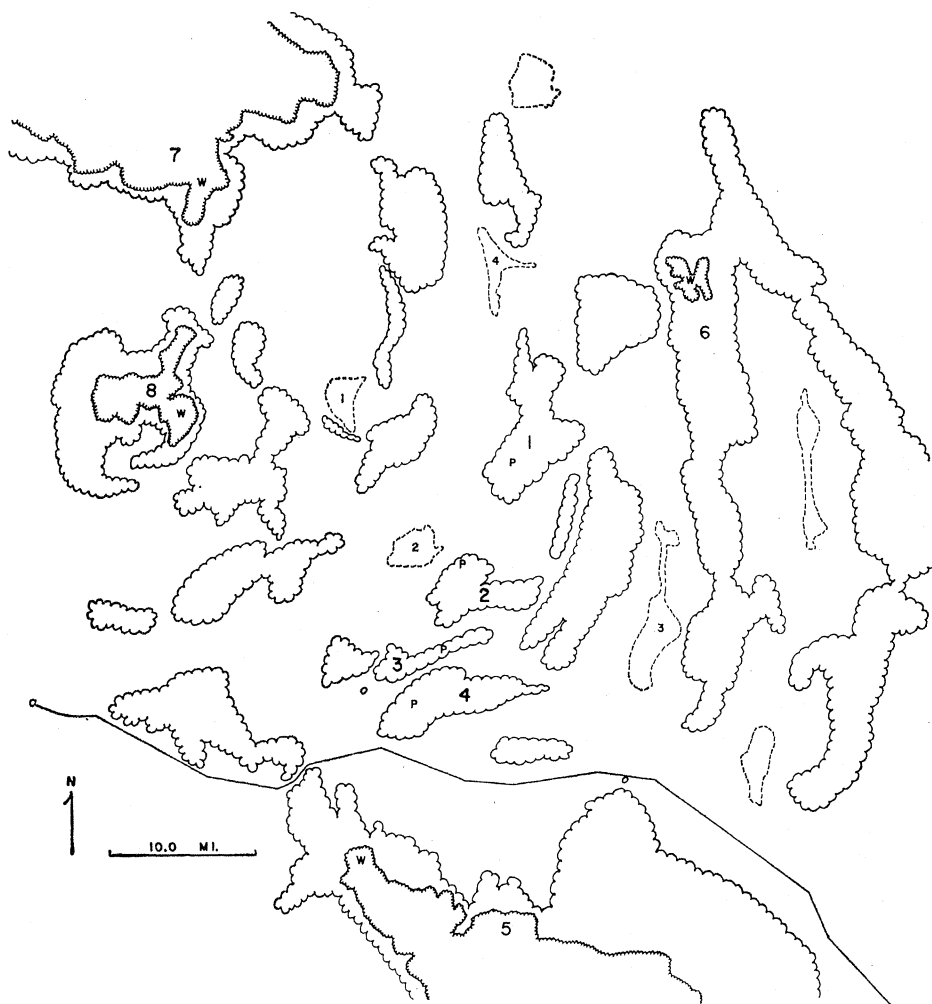


Fig. 1. Mountain ranges and playas in the region of Frenchman Flat, Nevada. Mountain ranges are outlined by crenate lines and designated by larger numerals, playas by dashed lines and smaller numerals. Extent of existing pinyon-juniper woodlands on higher mountains shown by more finely crenate lines. Highway U.S. 95 shown as a solid line. Symbols: *W*, existing pinyon-juniper woodland; *P*, Pleistocene midden of wood rat containing juniper twigs and seeds. 10 mi = 16.1 km.

Mountains	Maximum elevation (m)	Playas	Elevation (m)
I. Lacking pinyon-juniper woodland at present:		1. Yucca Flat	1193
1. Aysees Peak	1906	2. Frenchman Flat	939
2. Ranger Mtns.	1638	3. Indian Springs	945
3. Mercury Ridge	1608	4. Papoose	1393
4. Spotted Range	1853		
II. With existing pinyon-juniper woodland:			
5. Spring Range	3631		
6. Pintwater Range	2146		
7. Pahute Mesa	2351		
8. Shoshone Mtn.	2154		

plant constituents of abandoned middens of the wood rat (*Neotoma*) in the desert ranges around Frenchman Flat (Fig. 1). Radiocarbon dating of wood and other organic materials from eight *Neotoma* middens bearing juniper twigs and seeds suggests that the middens are from 7800 to greater than 40,000 years old (Table 1).

The limited foraging range of the wood rat assures that macrofossils preserved in their ancient middens repre-

sent relatively local vegetation. Therefore the middens probably contain more precise information on local paleoclimate than sediments yielding fossil pollen, because of the wide dissemination of many types of pollen, especially that of wind-pollinated conifers. Hence, *Neotoma* middens may have unique value as a check on the palynological approach to Pleistocene ecology in the arid Southwest.

The middens occur in shallow caves or rock shelters which are not infrequent in the Paleozoic limestones and dolomites of the mountains south and east of Frenchman Flat playa (Fig. 2). Most of the old middens are characterized by a peculiar varnish-like coating, consisting of lustrous, dark-brown

masses of dried urine of *Neotoma*. The exceptional thickness of this glossy substance and the lack of loosely strewn plant debris enable one to distinguish superficially the older middens from recently occupied "houses" of *Neotoma lepida*, which are common in the same area. Among the bulk constituents of the Pleistocene middens are compact masses of woody plant remains (chiefly twigs of *Juniperus*), fine earthy material (possibly aeolian silt), fecal pellets of *Neotoma*, and the cementing dried urine. Skulls and other bones of *Neotoma lepida* are present. Some of the larger deposits attain lateral dimensions of 150 cm, but rarely do they exceed 75 cm in thickness, and stratification is usually poorly developed. In a single small cave in the Ranger Mountains, several more or less discontinuous masses of ancient midden material were present. Each mass was less than 60 cm in maximum vertical thickness, and all were of similar composition. Nevertheless, radiocarbon dates of four samples indicate a range in age of about 19,000 years.

Many well-preserved twigs, leaves, seeds, and vertebrate bones are present in most deposits, which permit the positive identification of a number of species; these include several of climatic significance, notably Utah juniper (by far the most abundant species present) and single-leaf pinyon pine (Table 1). Most of the small mammals recorded, including the wood rat, range from woodland down to desert elevations at present. The marmot (*Marmota flaviventris*) is the only species noted in the ancient middens which no longer occurs in southern Nevada. The nearest existing populations are in the high mountains in the central part of the state centering on the Toiyabe Range, about 200 km north of Frenchman Flat (2).

All middens bearing juniper twigs were located within limits of elevation ranging from 1100 to 1830 m, a span currently vegetated by the *Larrea* and *Coleogyne* desert shrub zones. In fact, most of the deposits occur in a transition zone near the upper limits of *Larrea* and the lower limits of *Coleogyne* (1100 to 1550 m). From this distribution comes the inference that the entire existing *Coleogyne* zone and at least the upper part of the existing *Larrea* zone around Frenchman Flat were occupied by evergreen juniper woodlands during part of Pleistocene time.

The lower limit of living pinyon-

juniper (or juniper) woodland in the region under discussion is about 1700 m above sea level at present, but only on the higher mountains does woodland occur this low. The lowest Pleistocene midden containing juniper, so far discovered, is at 1100 m in the Ranger Mountains (Table 1). This represents a lowering of the woodland zone from its present position by about 600 m, as recently as about 10,000 years ago, or as long as about 29,000 years ago.

Both pinyon and juniper are lacking at present on Aysees Peak (maximum elevation 1906 m) and on ranges lower in elevation, but both survive on the Pintwater Range (maximum elevation 2146 m) and on ranges higher in elevation (Fig. 1). The present lowermost limits of woodland (about 1700 m) are observed only near mountains exceeding 2000 m, regardless of their maximum elevation. Hence it may be inferred that the present lowermost limits of pinyon-juniper woodland were formerly not only lower, but also (more recently) greater in elevation than at present, probably during the warm Hypsithermal climatic phase of post-Wisconsin time, beginning about 8000 years ago (3). An upward retreat adequate to eliminate pinyon or juniper from mountains lower than 2000 m elevation (for example, Aysees Peak) could still permit a later, local advance of pinyon-juniper (or juniper) wood-

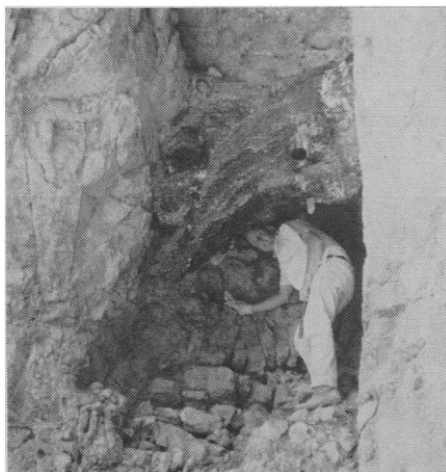


Fig. 2. Pleistocene midden of wood rat, directly above observer, in alcove-like shelter in limestone bedrock, Mercury Ridge, near Frenchman Flat, Nevada. Leafy twigs and seeds of an arborescent juniper (*Juniperus osteosperma*) are abundant in the midden, but the surrounding vegetation is a low desert scrub and junipers are totally lacking at present. Dried urine from the midden was estimated by radiocarbon dating to be more than 40,000 years old.

Table 1. Significant plant and animal fossil content and radiocarbon ages of wood rat middens located east or south of Frenchman Flat.

Elevation of midden site (m)	Significant fossils in midden	Radiocarbon age* of midden (yr)
Aysees Peak		
1525	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds) <i>Cercocarpus ledifolius</i> (few leaves)	9320 ± 300 (644)
Ranger Mountains		
1100	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds)	10,100 ± 160† (107) 17,450 ± 300 (555) 27,400 ± 800 (108) 28,900 ± 1200‡ (150)
1130	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds)	16,800 ± 300 (556)
Mercury Ridge		
1250	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds) <i>Marmota flaviventris</i> (skull)	12,700 ± 200† (561)
1280	<i>Juniperus osteosperma</i> (several leafy twigs)	7800 ± 150 (560)
1400	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds)	> 38,000 (558) > 40,000 (557)
1390	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds)	9000 ± 250 (559)
Spotted Range		
1550	<i>Pinus monophylla</i> (several leaves, cone scales, seeds) <i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds) <i>Bassarisctus astutus</i> (jawbone)	> 40,000† (151)
1830	<i>Juniperus osteosperma</i> (abundance of leafy twigs and seeds)	

\* Numbers in parentheses refer to UCLA series of radiocarbon dates, determined at Institute of Geophysics, University of California, Los Angeles. † Age estimated by radiocarbon dating of wood; all others by dating of uriniferous material. Based on organic residue after HCl treatment.

land from refugia on higher mountains downslope to present lower limits of about 1700 m. On the mountains immediately surrounding Frenchman Flat the lower limit of juniper woodland probably retreated upward the indicated span of about 800 m (that is, 1100 to 1906 m) prior to local extinction of the juniper here at some time during the past 9000 years.

Although pinyon pine needles are a conspicuous component of currently active wood rat deposits in the pinyon-juniper zone, it should be emphasized that remains of pinyon pine are absent from the ancient *Neotoma* middens below an elevation of 1550 m. Pinyon pine has been recorded in only one midden located at 1550 m in a canyon on the north side of the Spotted Range, where several seeds, cone-scales, and leaves of *Pinus monophylla* were detected in the basal portion of a deposit dated by radiocarbon as being more than 40,000 years old. Also, curl-leaf mountain mahogany (*Cercocarpus ledifolius* Nutt.), a species usually occurring in the upper pinyon zone or higher) has been found only at 1525 m on Aysees Peak, in a small midden which is about 9300 years old. On the other hand, twigs and seeds of Utah juniper are very abundant in most of the de-

posits located at or above 1100 m. The Utah juniper occurs regularly at lower elevations than the pinyon pine on the higher mountains of this region at the present time, and it is evidently more xerophytic. Hence it follows that in ancient *Neotoma* midden time (from 7800 to more than 40,000 years ago) the climatic conditions between 1100 and 1500 m on the low ranges in the vicinity of Frenchman Flat were significantly less arid than at present, but probably more arid than in the usual pinyon-juniper environment which probably occurred only above 1500 m (4).

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#### References and Notes

1. The principal desert shrub zones of the northern Mohave Desert pertinent to this study are the *Larrea* and *Coleogyne* zones. The former zone occupies the lower elevations and is dominated by widely spaced individuals of the evergreen creosote bush (*Larrea divaricata* Cav.), about 100 cm in height. The intervening spaces are sparsely covered by shrubs of lesser stature, one of the most important being white bur-sage (*Franseria dumosa* Gray). The *Coleogyne* zone overlaps the upper elevation range of *Larrea* but extends to higher elevations as nearly pure stands of the evergreen blackbrush (*Coleogyne ramosissima* Torr.) and ranges into the lower pinyon-

- juniper woodland zone. The shrubs are more closely spaced than is usual for *Larrea*, but average only about 40 cm in height [P. V. Wells, *Ecology* 41, 553 (1960)].
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  - C. J. Heusser, *Am. Geographical Soc. Spec. Publ. No. 35* (1960).
  - A fair indication of relative annual precipitation and temperature regimes in the pinyon-juniper, *Coleogyne* and *Larrea* zones in Nevada, Utah, Arizona, and California is afforded by records of meteorological stations situated in the respective zones [*Climate and Man*, U.S. Department of Agriculture Yearbook (Government Printing Office, Washington, D.C., 1941)]. The following is a comparison of mean annual precipitation in the three zones: Pinyon-juniper zone (20 stations): range, 26.4 to 42.4 cm; mean, 33.3 cm. *Coleogyne* zone (9 sta-

- tions); range, 11.4 to 32.3 cm; mean, 20.6 cm. *Larrea* zone (20 stations): range, 6.4 to 22.1 cm; mean, 12.4 cm. The differences in precipitation among zones are reinforced by differences in temperature, the pinyon-juniper zone (highest in elevation) being coolest, the *Larrea* zone (lowest in elevation) warmest, and the *Coleogyne* zone intermediate.
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amino acids into protein and the effect of polynucleotides thereon being in general quite small. It was therefore deemed of interest to examine microorganisms other than *E. coli*.

*Alcaligenes faecalis* was chosen because its DNA differs significantly in base composition from that of *E. coli*. The polynucleotides used were polyA, polyC, and polyU as homopolymers; polyAC (5:1), polyAG (5:1), and polyAU (5:1) as A-rich copolymers; and polyUA (5:1), polyUC (5:1), and polyUG (5:1) as U-rich copolymers. Eighteen triplets of the base composition indicated could be assigned to the following 13 amino acids: arginine, 2A1C; cysteine, 2U1G; glutamic acid, 2A1G; lysine, AAA; phenylalanine, UUU; proline, CCC, 2C1A, 2C1U; serine, 2U1C; threonine, 2A1C; tyrosine, 2U1A; and valine, 2U1G. Comparison with the data on *E. coli* (6) shows identical assignments. Thus, as far as the data go there is agreement between *A. faecalis* and *E. coli*. About as many A- and U-containing triplets specify amino acids in *A. faecalis* (DNA, 66 percent GC) as in *E. coli* (DNA, 52 percent GC). However, the difference in GC content between the DNA's of these two organisms is hardly large enough to expect significant differences in this regard. The present survey, although more extensive than previous ones in animal tissues, was not broad enough to rule out the occurrence of coding units in *A. faecalis* differing in amino acid specificity from

## Amino Acid Code in *Alcaligenes faecalis*

**Abstract.** Amino acid incorporation into protein, promoted by synthetic polynucleotides, was studied in a cell-free extract of *Alcaligenes faecalis* (combined guanine and cytosine content of DNA, 66 percent). In the limited survey, 18 code triplets were found to specify the amino acids as in *Escherichia coli* (combined guanine and cytosine content of DNA, 52 percent). At least six of the eight possible triplets containing adenine and uracil were meaningful. No exceptions to the universality of the amino acid code were found.

The base composition of the coding units of messenger RNA (1) has been studied by using polyribonucleotides of widely varying base composition as artificial messengers in a cell-free system of *Escherichia coli*. More than 40 code triplets have been assigned to the 20 amino acids (2), and the list is probably incomplete. These results suggest extensive degeneracy of the amino acid code. There are indications that the code is universal (3). These indications received some support from stud-

ies with cell-free systems derived from mammalian cells and organisms other than *E. coli* (4, 5). In general, polyA codes for lysine, polyU for phenylalanine, and polyUA, polyUC, and polyUG have coding units for phenylalanine, isoleucine, and tyrosine, for phenylalanine and serine, and for phenylalanine and valine, respectively, as in *E. coli*. In the case of animal tissues and yeast a more extensive survey is precluded by the low activity of the available systems, the incorporation of

Table 1. Amino acid incorporation in the *A. faecalis* system with various polynucleotides.\* The amount of polynucleotide added (+) was 80  $\mu$ g/ml except for polyC where the amount was 400  $\mu$ g/ml, and for polyUA, polyUC, and polyUG where the amount was 160  $\mu$ g/ml. The minus signs indicate no addition (control).

Amino acid	Polynucleotide																	
	A		C		U		AC (5 : 1)		AG (5 : 1)		AU (5 : 1)		UA (5 : 1)		UC (5 : 1)		UG (5 : 1)	
	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
Alanine													60	50				
Arginine									30	190			10	10				
Asparagine					40	50	20	365			29	153	29	118	40	30	40	30
Aspartic acid					70	80	27	639			30	153	38	168	70	50	70	60
Cysteine													70	110			140	799
Glutamic acid	64	73	36	45	11	15	20	40	20	150			40	30				
Glutamine							14	252	20	20			14	10				
Glycine					20	10							20	30			24	339
Histidine							220	240					30	10				
Isoleucine											21	156	29	639				
Leucine					80	430					74	89	83	509	57	2170	34	1083
Lysine	64	1750			20	10	52	2600	60	770	54	1190	9	49				
Methionine											42	36						
Phenylalanine					182	50,600							53	3120	118	12,630	44	4415
Proline			9	156			10	140					10	10	32	516		
Serine													20	50	49	2120		
Threonine							25	1064			40	30	20	20				
Tryptophan													230	220			139	731
Tyrosine											71	96	38	695				
Valine													28	28	42	35	15	1180

\*  $\mu$ mole/mg ribosomal protein. The values with copolynucleotides are averages of experiments carried out in duplicate.