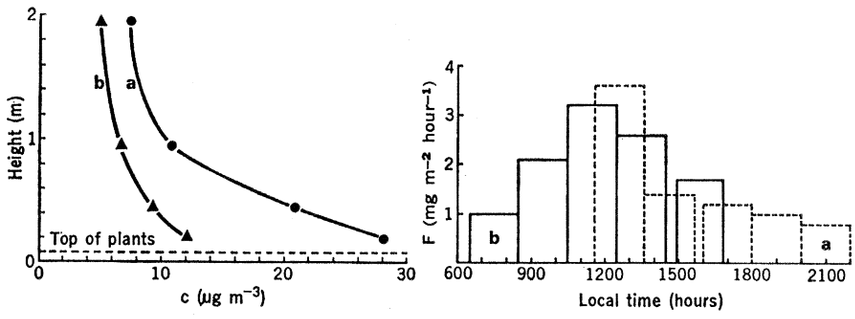

Fig. 1 (left). Profiles of ammonia concentration, c, over an alfalfa pasture: profile a, 1600–1800 hours on 14 March 1974; profile b, 1500–1700 hours on 15 March 1974. Fig. 2 (right). Flux densities of nitrogen (as ammonia and related compounds), F, from pasture: profile a, 14 March 1974; profile b, 15 March 1974.

used to calculate h from Eq. 1. Determination of the ammonia concentration by our absorption method prevented on-the-spot monitoring, and, because of our uncertainty about the concentrations that were likely to prevail from time to time, the sampling periods for this measurement were longer, 1 or 2 hours. Appropriate averages of h were then used to calculate F from Eq. 2.

There is some ecological interest in the atmospheric concentrations of ammonia and in its flux density. In 28 sampling measurements the average concentration at 0.20 m was 15.7 μg m^{-3} with a range of 3.4 to 51.5 μg m^{-3}. In the same measurements the average concentration at 0.95 m was 10.1 μg m^{-3} with a range of 1.6 to 28.4 μg m^{-3}. Two representative profiles measured at about the same hour on consecutive days are shown in Fig. 1. The very large differences in concentration and gradient were due to differences in the turbulence of the air. Profile a, for instance, was measured in light winds, about 0.9 m sec^{-1} at 1 m above the ground, whereas profile b was measured in strong winds, about 3 m sec^{-1} at the same height. The flux densities of ammonia were similar, however, at about 1.5 mg m^{-2} hour^{-1}. Obviously, little information can be obtained about the magnitude of ammonia exchange from measurements of concentration alone [see, for example, the procedure of Hutchinson and Viets (*2*)].

The general character of the flux determinations is shown in Fig. 2. Those observations were made during a period of 29 hours covering the daylight period and some of the night. A diurnal cycle in the flux is clearly evident with peak flux densities of nitrogen (in excess of 3 mg m^{-2} hour^{-1}) occuring around midday and minimum values (about 0.8 mg m^{-2} hour^{-1}) overnight. Twenty-six calculations were made between 7 March and 29 March on seven separate days. These gave an average daily loss of nitrogen of 0.26 kg ha^{-1}.

Our investigations are still at a preliminary stage, and many aspects remain to be studied, including the seasonal cycle of ammonia exchange. Nonetheless, it is apparent from these measurements that losses of gaseous nitrogen compounds can constitute a large part of the nitrogen turnover in a grazing system. The input of nitrogen to leguminous pastures by symbiotic nitrogen fixation varies with plant species and growing conditions, but annual amounts between 100 and 200 kg ha^{-1} have often been reported (*8*). In addition, rain adds small quantities of nitrogen which we estimate at about 5 kg ha^{-1} year^{-1}. Of these, about 50 kg ha^{-1} remain in the soil (*9*), and the equivalent of about 20 kg ha^{-1} remain in the animals. Thus the annual loss of nitrogen from the system is often of the order of 100 kg ha^{-1} or an average of 0.27 kg ha^{-1} day^{-1}. The almost exact coincidence of this figure with our estimates of ammonia loss must be regraded as fortuitous, but the calculations certainly establish the importance of gaseous losses in the nitrogen economy of grazing systems. In the situation we studied the sheep were returning an estimated 1 kg of nitrogen per hectare per day as urine, which could have been a major source of the observed flux of ammonia (*5*). The data suggest that grazed pastures are important contributors of ammonia nitrogen to the atmosphere and, through it, to other land areas.

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17 April 1974

Photosynthetic Mechanisms and Paleoecology from Carbon Isotope Ratios in Ancient Specimens of C_4 and CAM Plants

Abstract. *Carbon isotope ratios of modern, 10,000-year-old, and more than 40,000-year-old* Atriplex confertifolia *(C_4) material from Nevada caves indicate that the C_4 photosynthetic pathway was operating in these plants over that period. Samples of a plant with crassulacean acid metabolism,* Opuntia polyacantha, *were also measured, and a shift in the $\delta^{13}C$ value from −21.9 per mil (more than 40,000 years ago) to −13.9 per mil (10,000 years ago) was observed. This provides unique physiological evidence to support the hypothesis that the late Pleistocene pluvial climate in the region already had become drier about 10,000 years ago.*

The carbon isotope ratio technique has been used in paleoecology to follow the photosynthetic pathways in plants throughout geological time (*1*). The proposal that it would be useful to study the past geographical distribution of C_3 and C_4 plants (*1*) has been limited by the scarcity of C_4 remains, but a series of dry caves in the Mohave Desert sector of southern Nevada, western North America, provides this material with ages spanning the past

Table 1. Radiocarbon ages and carbon isotope ratios of present-day and ancient leaves and fruits of *Atriplex confertifolia*.

Site	Age (years)	$\delta^{13}C$ (per mil)
South of Pyramid Peak, California	< 5	−14.8
Ranger Mountains, Nevada	10,000 ± 160	−16.2
Mercury Ridge, Nevada	> 40,000	−13.4

40,000 years (*2–4*). The $\delta^{13}C$ values of modern, 10,000-year-old, and > 40,000-year-old subfossil deposits of *Atriplex confertifolia* (T. & F.) Wats. from these caves have been measured (*5*).

To further investigate photosynthetic mechanisms responsible for carbon accumulation in previous climatic periods, carbon isotope ratios were determined for a plant with crassulacean acid metabolism (CAM), a prickly pear cactus (*Opuntia polyacantha* Haw.) present in the same caves. These measurements were of special interest because it has been shown that the carbon pathway and carbon isotope ratio in some CAM plants can be manipulated by environmental features such as night temperature, salinity, day length, and water stress (*6, 7*). Consequently, gross changes in the environment during geological time may influence the relative importance of the C_3 or CAM nature of the photosynthetic mechanisms of *O. polyacantha*.

Significantly different environments are indicated by the two ancient *Neotoma* (pack rat) middens of different age from near Frenchman Flat, Nevada, which contain the *Opuntia* and *Atriplex* material used in our study. The difference is superficially obscured because both deposits show a predominance of the woodland conifer (C_3), *Juniperus osteosperma*, and thus document pluvial conditions dramatically cooler and moister than the extremely arid desert climate that now prevails at these and other sites at low elevation throughout the Mohave Desert region (*3*). However, the deposit with an indeterminate radiocarbon age of > 40,000 years (UCLA 557) has a very low representation of the xerophytic CAM and C_4 plants (*Opuntia* and *Atriplex*), but contains abundant remains of a C_3 plant, the cliff rose (*Cowania mexicana*), a characteristic woodland shrub. In contrast, the 10,000-year-old deposit (UCLA 160) has a relatively abundant content of *Opuntia* and *Atriplex* but lacks the cliff rose. The substantial quantitative difference in floristic composition between the two deposits suggests a cooler or wetter climate for the older record. It is also of special interest that the reduction in the abundance of the C_3 plant cliff rose was associated with increasing aridity.

On the basis of this climatic change it can be predicted that the $\delta^{13}C$ value of CAM plants could alter over the same period, from a more C_3-like value (−27 per mil) to a more C_4-like number (−13 per mil) (*8*). The results test this prediction.

It has previously been shown that two carbon pathways occur within the *Atriplex* genus (*9, 10*) and that the carbon isotope ratio technique distinguishes between C_3 and C_4 members of this genus (*9, 11*). From the $\delta^{13}C$ value (*12*) for the present-day sample (Table 1) it is evident that *Atriplex confertifolia* is a C_4 plant. The $\delta^{13}C$ values of −16.2 per mil for the 10,000-year-old sample and −13.4 per mil for the > 40,000-year-old sample (Table 1) confirm our expectations that this *Atriplex* species was using the C_4 pathway > 40,000 years ago. The results also show there were no other sources of carbon contaminating the samples.

For the CAM plant, *Opuntia polyacantha*, the measured $\delta^{13}C$ values were −21.9 per mil for a > 40,000-year-old sample and −13.9 per mil for a 10,000-year-old sample (Table 2). This shift in the $\delta^{13}C$ value from a C_3-like to a C_4-like number is consistent with the prediction that the climatic change over this period would induce a change in the photosynthetic mechanism in CAM plants.

The C_4-like carbon isotope ratios in CAM plants have been interpreted as indicating both a capacity for dark CO_2 fixation involving phosphoenolpyruvate (PEP) carboxylase and a lack of significant CO_2 exchange in the light over long periods. Several environmental features may influence the CAM behavior of the plants in natural environments. Night temperature may enhance acid formation in the dark and may influence CO_2 exchange in the light, but measurements of the influence of temperature on the $\delta^{13}C$ value of four CAM plants indicated a small change (3 per mil) over the range 15° to 40°C (*13*).

Water stress was shown to be a significant factor in influencing the carbon isotope ratio in a *Kalanchoe* species (*6*). It may act to increase dark CO_2 fixation and reduce CO_2 exchange in the light through an effect on stomatal resistance. The water-use efficiency of CAM plants compared to C_3 and C_4 plants would be maximized under conditions of high stomatal resistance—and therefore low CO_2 and H_2O vapor exchange (*14*)—during the light period, when potential evaporation is highest because of more radiation, higher temperatures, and lower specific humidity. High water-use efficiency is achieved by taking up CO_2 at night by the PEP carboxylase reaction and maintaining a relatively closed system in the light during transfer of carbon from the C_4 acids to the C_3 pathway. The same conditions favor minimal isotope fractionation during carboxylation because PEP carboxylase is involved. This is further supported by the high correlation between dark CO_2 fixation, C_4-like $\delta^{13}C$ values, and aridity of the site established in an investigation of photosynthetic behavior in the Bromeliaceae (*15*). In this respect, the carbon isotope ratio in CAM plants may be used as an indicator of the water regime of a site.

Table 2. Radiocarbon ages and carbon isotope ratios of ancient samples of the CAM plant *Opuntia polyacantha*.

Site	Age (years)	$\delta^{13}C$ (per mil)
Ranger Mountains, Nevada	10,000 ± 160	−13.9
Mercury Ridge, Nevada	> 40,000	−21.9

The radiocarbon dating of former vegetation preserved in cave deposits over much of the Mohave Desert documents a major shift in climate from relatively cool, moist, woodland conditions to an extremely warm, arid, desert environment after about 9000 years ago. Deposits older than 9000 years consistently show abundant remains of woodland conifers, whereas deposits younger than about 8000 years contain a record of warm desert scrub. The vegetational change corresponds to a temperature increase of roughly 5°C, and a decrease in annual precipitation of perhaps 20 cm in going from full-glacial woodland to postglacial desert (*4*). On the other hand, climatic changes within the pluvial woodland period from 9,000 to > 40,000 years ago are less obvious. The vegetational differences between the deposits 10,000 and > 40,000 years old located near Frenchman Flat are significant, but indicate a degree of

climatic change perhaps an order of magnitude less than the difference between full-glacial, pluvial woodland and postglacial, desert conditions.

Assuming that the $\delta^{13}C$ values of the samples > 40,000 and 10,000 years old are indicative of their photosynthetic mechanism and water-use efficiency, then these results provide further evidence of increasing dryness of the Nevada site. The presence of the C_4 species of *Atriplex* at both times is evidence that conditions throughout the entire period are likely to have been relatively arid. Intracellular resistance to CO_2 exchange is lower in C_4 plants than C_3 plants, which is apparently responsible for the higher water-use efficiency in C_4 plants (*10*). The evidence that the C_4 species, *Atriplex confertifolia*, was much more abundant in the 10,000-year-old deposit can be taken as an indication of especially warm and dry conditions toward the close of the last major glacial stage of the Pleistocene. Presumably, the shift from C_3-like to C_4-like carbon isotope ratios in the CAM plant (*Opuntia polyacantha*), also indicates that the Frenchman Flat area was already more arid about 10,000 years ago, even a millennium or more before the local demise of the pluvial juniper woodlands.

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$$\delta^{13}C \text{ (per mil)} = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{PDB}} - 1\right] \times 1000$$

where the PDB standard is the belemnite from the Peedee Formation in South Carolina.
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12. The leaf material was collected from cave deposits and carbon dated by using procedures described by Wells and Berger (*3*). There is a possibility of some contamination of the sample by carbon from other sources, but in the work reported here there was no pretreatment of the sample, and the results suggest that it may not be essential for the object of this study. The material was burned to completion in an oxygen atmosphere and all CO_2 was recovered free from contamination. The $^{13}C/^{12}C$ ratio was measured in a mass spectrometer, with appropriate corrections for effects of other isotopes on mass 44 and 45 and instrument effects. The value was expressed as $\delta^{13}C$; an accuracy of ± 0.1 per mil is expected.
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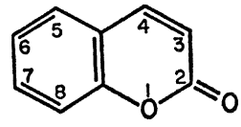
Genetic Variation in Coumarin Hydroxylase Activity in the Mouse (Mus musculus)

Abstract. *Basal and phenobarbital-induced rates of hepatic metabolism of coumarin to 7-hydroxycoumarin are markedly higher in DBA/2J mice than they are in the AKR/J, C57BL/6J, and C3H/HeJ strains. Intermediate coumarin hydroxylase activity in F_1 hybrids of mating between DBA/2J and the other three strains indicates an additive mode of inheritance.*

Studies with different strains of the same animal species (*1*) and family studies in man (*2, 3*) indicate significant genetic regulation of microsomal mixed-function oxidases that metabolize drugs, carcinogens, and other foreign chemicals (*4*). Working with inbred strains of mice, Thomas *et al.* (*5*) and Nebert and his associates (*6*) have shown that induction of benzo[*a*]pyrene hydroxylase (aryl hydrocarbon hydroxylase) by 3-methylcholanthrene and other polycyclic hydrocarbons is principally determined by a single autosomal dominant gene. We now describe genetic control of basal and phenobarbital-induced levels of a microsomal mixed-function oxidase that hydroxylates coumarin (see structure), a naturally occurring constituent of many plants and the nucleus for the coumarin oral anticoagulant drugs.



Coumarin

Male mice of four inbred strains and three F_1 hybrids, 6 to 7 weeks old, were purchased from the Jackson Laboratory, Bar Harbor, Maine. Hepatic metabolism of coumarin to 7-hydroxycoumarin was determined spectrofluorometrically by a modification of the method of Creaven *et al.* (*7*). Incubation mixtures, in a final volume of 1.0 ml, contained 2 mg (wet weight) of liver, 50 μmole of tris-HCl (*p*H 7.5), 250 μmole of sucrose, 3 μmole of $MgCl_2$, 0.5 μmole each of the reduced forms of nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH), and 1.0 μmole of coumarin.

Basal and induced coumarin hydroxylase activities in four inbred mouse strains and three F_1 hybrids are summarized in Table 1. The data indicate that enzyme activity in noninduced DBA/2J mice is three- to fourfold higher than in AKR/J, C57BL/6J, or C3H/HeJ mice. After 3 days of phenobarbital treatment, the relative difference in enzyme activity between DBA/2J and the other three strains is four- to sixfold. 7-Hydroxycoumarin formation in liver homogenates of F_1 hybrids of matings between DBA/2J mice and the other three strains is intermediate in both untreated animals and those treated with phenobarbital, indicating additive inheritance. Table 1 also shows the lack of induction of coumarin hydroxylase activity by 3-methylcholanthrene (3-MC) in any of the inbred or hybrid mice. In agreement with published results (*5, 6*) benzo[*a*]pyrene hydroxylase activity in liver homogenates from the same ani-