mum potential size in the species, however, appears to be impossible without tocopherol. The adaptive significance, if any, of the α-tocopherol requirement for the extreme size of campanulates is enigmatic.

The inhibition of mictic female production in campanulates may be extremely adaptive, for it would allow continuous, rapid, parthenogenetic reproduction at times when the diet contains large, as well as tocopherol-rich, prey. Otherwise, production of males and resting eggs, which may be dormant for a long time, would decrease the reproductive rate when such food was abundant. Since α-tocopherol is found in most algae (*20*) and hence in algivorous zooplankters, the diet available to this species often must be tocopherol-rich. Inhibition of sexuality in campanulates would not endanger the long-term survival of the population by resting eggs, because natural populations are not composed entirely of campanulates (*4, 5, 7*) and because sexual cruciforms would be produced before, and possibly after, campanulate production.

JOHN J. GILBERT
Department of Biological Sciences,
Dartmouth College,
Hanover, New Hampshire 03755

References and Notes

1. C. W. Birky, Jr., and J. J. Gilbert, *Amer. Zool.* **11**, 245 (1971); J. J. Gilbert, *Limnol. Oceanogr.* **16**, 309 (1971).
2. E. v. Daday, *Math. Naturwiss. Ber. Ungarn* **7**, 140 (1888); P. de Beauchamp, *Bull. Biol. Fr. Belg.* **85**, 137 (1951); H. Kiechle and H. Buchner, *Res. Suisse Zool.* **73**, 283 (1966).
3. A. Lange, *Zool. Anz.* **38**, 433 (1911).
4. J. H. Powers, *Amer. Natur.* **46**, 441 (1912).
5. C. W. Mitchell, *J. Exp. Zool.* **15**, 91 (1913).
6. J. J. Gilbert and G. A. Thompson, Jr., *Science* **159**, 734 (1968).
7. S. H. Hurlbert, M. S. Mulla, H. R. Willson, *Ecol. Monogr.* **42**, 269 (1972).
8. The cruciform morphotype has four outgrowths of the body wall—two lateral, one posterodorsal, and one posterior.
9. L. A. Riggs and J. J. Gilbert, *Int. Rev. Ges. Hydrobiol.*, in press.
10. J. J. Gilbert and C. W. Birky, Jr., *J. Nutr.* **101**, 113 (1971).
11. J. J. Gilbert, *Physiol. Zool.* **41**, 14 (1968).
12. This treatment preserves the rotifers in an expanded condition [C. W. Birky, Jr., and J. A. Power, *J. Exp. Zool.* **170**, 157 (1969)].
13. A. C. Giese, *Trans. Amer. Microsc. Soc.* **57**, 245 (1938).
14. Distillation Products Industries, courtesy of Dr. D. C. Herting.
15. C. W. Birky, Jr., *J. Exp. Zool.* **169**, 205 (1968).
16. C. W. Birky, Jr., unpublished observations.
17. Saccates probably incorporate a large proportion of emulsified α-tocopherol by ingestion of paramecia intermediates, absent from these cultures.
18. U. Halbach, *Zool. Anz.* **33** (Suppl.), 72 (1970); *Mikrokosmos* **10**, 289 (1971).
19. J. J. Gilbert, *Science* **151**, 1234 (1966); *Arch. Hydrobiol.* **64**, 1 (1967).
20. W. A. Skinner and P. A. Sturm, *Phytochemistry* **7**, 1893 (1968); E. Sun, R. Barr, F. L. Crane, *Plant Physiol.* **43**, 1935 (1968).
21. I thank M. Bean for technical assistance and H. L. Allen, C. W. Birky, Jr., D. S. Dennison, R. T. Holmes, S. H. Hurlbert, for reading and improving the manuscript. Supported by NSF research grant GB-31282.

23 March 1973 ■

Fire and the Nitrogen Cycle in California Chaparral

Abstract. *Analysis of soils from burned and unburned chaparral indicates that high nitrate concentrations following fire are due to the addition of ammonium and organic nitrogen in the ash. Inhibition of mineralization in unburned chaparral results in low nitrate concentrations. Fluctuations in the amount of soil nitrate in unburned chaparral are the direct result of foliar leaching.*

Fire is a frequent occurrence in chaparral, a shrubby vegetation common in areas subject to prolonged summer drought. Many recent studies have emphasized the beneficial aspects of such brush fires in the maintenance of a healthy plant cover (*1*). One such benefit is the addition to the soil of ash which has high concentrations of nutrients that were previously held in the standing crop. Nitrate has been of particular interest in this regard because of its apparent deficiency in many chaparral soils (*2*) and comparatively high concentrations after burning (*3*). Although speculation is plentiful, the questions of why chaparral soils are low in nitrate and why nitrate concentrations increase after fire have largely been left unanswered.

The data presented here are part of a study undertaken to investigate changes in various essential plant nutrients after fire and to evaluate their importance in revegetation after fire. In September 1971 a 30-ha plot of dense chaparral, dominated by *Adenostoma fasciculatum* (chamise), burned in the Santa Ynez Mountains near Santa Barbara, California. Because of its close proximity to unburned chaparral growing on the same soil type and slope, this area was ideal for study. Soil samples were collected immediately after the burn and at nine other times through the next 14 months. On each date six 1-kg samples of the upper 2 cm of mineral soil were taken from each of the burned and unburned chaparral. After being passed through a 2-mm screen, each sample was analyzed for organic matter content, total nitrogen, exchangeable ammonium, and exchangeable nitrate (*4*). The *p*H of each sample in water was also determined (*4*). Soil samples were also collected in the field in airtight tins for moisture analysis. To evaluate the effects of shrub removal without the addition of ash, clearings were made in mature chaparral in the late summer of 1971 and of 1972. Soil from these clearings was analyzed in the same manner as that from the burned and mature chaparral areas. Statistical comparisons were made with the use of Student's *t*-test. In this report all soil nutrient values are expressed relative to soil oven-dry weight.

To assess the importance of foliar leaching of nutrients in unburned chaparral, several rain-collection vessels were placed under the shrub cover and in the cleared areas in the fall of 1972. These vessels were fitted with a float valve allowing the collection of a maximum of 0.6 cm (0.25 inch) of rainwater from any single storm. As materials washed from plant leaves would be concentrated in the first few milliliters of rain drip, this valve arrangement prevented dilution and aided quantification. Collected rainwater was returned to the laboratory as quickly as possible for analysis.

The total content of organic matter in unburned chaparral soil varied between 12 and 24 percent, but constituted only 6 to 8 percent of the burned-over soil. The total nitrogen content was also lower in the burned soil than in the unburned soil (2.7 versus 3.0 mg of nitrogen per gram of soil), but this apparent loss was not proportional to the loss in total organic matter.

The aboveground parts of several *Adenostoma* shrubs were incinerated, and the ash was analyzed in the same manner as the soil. The nitrate content was comparatively low in the ash (1.3 μg of nitrogen per gram), whereas the ammonium content was high (127 μg of nitrogen per gram). The total nitrogen content was 7100 μg/g. Ignition of this ash at 700°C resulted in a 38 percent loss, indicating a high content of organic matter. It is clear that ash not only supplies mineral nitrogen to the soil but may also provide a reservoir of organic nitrogen.

Soil nitrate concentrations were nearly equal in the burned and unburned chaparral soil immediately after the fire (Fig. 1). Ammonium concentrations were, however, considerably higher in the burned soil than in the unburned soil and remained high throughout the study. After the first rain in October 1971, the soil nitrate concentration rose slightly in the burned area. Because the ash layer in the burn inhibited the penetration of water, it was not until the second rain, in November, that this soil was thoroughly wetted. Samples taken after this second rain had much higher nitrate concentrations. With the exception of a sharp drop after an atypically heavy rainstorm in late December 1971, nitrate concentrations remained high through the remainder of the study. From April to November 1972 nitrate concentrations in the soil of the burned area showed a steady decline. These concentrations are being monitored to determine if this trend continues.

The high nitrate concentrations in the burned-over soil are undoubtedly due to the nitrification of abundant ammonium and are not the result of direct addition in the ash. Ammonium concentrations remained high throughout the sampling period owing to the reservoir of organic nitrogen available for mineralization. This process was probably aided by slightly higher *p*H values in the burned area (6.6 versus 6.1) which tend to favor bacterial growth.

Nitrate concentrations in unburned chaparral were comparatively low most of the year with the exception of rather striking increases after the first autumn rain in both 1971 and 1972. While showing more variation, the soil ammonium concentration was highest in September prior to the rains and decreased after the first precipitation.

This inverse relation between ammonium and nitrate in unburned chaparral soil would seem, at first glance, to implicate the nitrification process. Several workers have noted that soil drying results in the release of free ammonium and amino acids prior to the addition of moisture in agricultural soils (*5*, *6*). Birch (*6*) has emphasized the importance of this phenomenon in areas of intense summer drought. The increase in the ammonium concentration through the dry months is undoubtedly the result of this effect. Because of the dependence of nitrifying bacteria on moisture, it is tempting to hypothesize that increases in the nitrate concentration after the first autumn rains are due to the increased activity of the nitrifying bacteria as a result of more favorable growing conditions. This would also explain the concurrent decline in ammonium.

If the above hypothesis were true, similar changes should have been observed in cleared but unburned chaparral soils. Although late summer ammonium concentrations in these soils were equal to those under the shrubs, the nitrate concentration showed no increase after the first October rains.

Analysis of the rainwater collected under the shrubs revealed 40 to 50 μg of nitrogen as nitrate per milliliter of rainwater in the first 0.6 cm of rain. Rainwater collected in the clearings contained no nitrate. Subsequent rain collections under the shrubs yielded much lower nitrate concentrations. This initial addition of nitrate by foliar leaching can easily account for the observed increases in nitrate in the soil. Tukey (*7*) has demonstrated that a variety of minerals may be leached from plants by rainwater. This foliar leaching of nitrate closely follows the leaching of toxins observed by McPherson and Muller (*8*). Materials tend to accumulate in and on the leaves through the dry season, being fairly concentrated in the first rain drip. Subsequent rains carry much less material from the leaves. The decreased addition from the shrubs and the high mobility of the nitrate ion in the soil result in low concentrations of nitrate through the rest of the year. Although nitrification almost certainly occurs in chaparral soils, the fluctuations in the nitrate content appear to be little, if at all, related to this process.

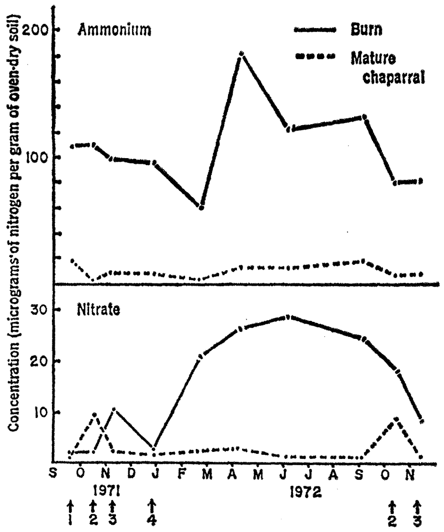

Fig. 1. Seasonal changes in the exchangeable ammonium and nitrate concentrations in burned and unburned chaparral soils: point 1, date of the fire, 17 September 1971; points 2 and 3, first and second rain of each season, respectively; point 4, atypically heavy rain in late December 1971. The values for the ammonium content of the burned soil were significantly different from those for the unburned soil ($P = .01$) at every sample date. Nitrate concentrations were similarly different at every date except those for the initial sample and the late December 1971 sample after the heavy rain.

The high total nitrogen and low mineral nitrogen (ammonium and nitrate) concentrations indicate inhibition of mineralization in unburned chaparral soil. The cause of this inhibition is not clear, but it may be the result of one or both of two processes. One possibility is that toxins present in chaparral soil may be inhibiting the activity of heterotrophic bacteria and fungi. Such toxins could be leached from the shrubs in rainwater or produced in the process of litter decomposition. A second possibility relates to the sclerophyllous leaf form common to many chaparral shrubs. Such leaves contain large quantities of lignin. Peevy and Norman (*9*) have demonstrated that high lignin content in litter resulted in greater resistance to decomposition and to the subsequent mineralization of nitrogen in agricultural soils. The proportion of lignin in chaparral soils would certainly be much higher than in most agricultural situations. This effect may be the result of a general resistance of soil lignin compounds to decomposition, especially in summer drought regions, or to the potential inhibitory nature of certain lignin decomposition products.

The data presented here should serve to caution ecologists involved in nutrient studies. The time of year and the frequency with which soil samples are taken may greatly affect the results obtained and the conclusions reached concerning soil fertility. Moreover, the importance of foliar leaching needs to be assessed in every case. These results would also seem to cast a great deal of doubt on the adequacy of total nitrogen and total organic matter content as indices of soil fertility in some plant communities. The value of such determinations rests on the susceptibility of these materials to decomposition.

NORMAN L. CHRISTENSEN
University of California,
Santa Barbara 93106

References and Notes

1. C. H. Muller, R. B. Hanawalt, J. K. McPherson, *Bull. Torrey Bot. Club* **95**, 225 (1968); H. A. Mooney and E. L. Dunn, *Evolution* **24**, 292 (1970); T. L. Hanes, *Ecol. Monogr.* **41**, 27 (1971).

2. J. Vlamis and K. D. Gowans, *J. Range Manage.* **17**, 73 (1961).
3. A. W. Sampson, *Calif. Agr. Exp. Sta. Bull. 635* (1944); J. R. Sweeney, *Univ. Calif. Publ. Bot.* **28**, 143 (1956); N. L. Christensen and C. H. Muller, in preparation.
4. I carried out the organic matter determinations by using the loss-on-ignition method given in F. E. Bear, Ed., *Chemistry of the Soil* (Reinhold, New York, ed. 2, 1964), pp. 475–476. I determined the total nitrogen content on Kjeldahl digests as described in M. L. Jackson, *Soil Chemical Analysis* (Prentice-Hall, Englewood Cliffs, N.J., 1958), using colorimetric techniques as given by J. Tetlow and A. Wilson, *Analyst* **89**, 453 (1964). Methods for the determination of exchangeable ammonium and nitrate are given by J. M. Bremner, in *Methods of Soil Analysis*, C. A. Black *et al.*, Eds. (American Society of Agronomy, Madison, Wis., 1965), vol. 2, pp. 1179–1227. I determined the nitrate content by means of the phenoldisulfonic acid method, and the ammonium content was determined by microdiffusion techniques. I also double-checked the ammonium concentrations, using the colorimetric analysis given by Tetlow and Wilson. Soil *p*H in water was determined by means of a glass electrode in a 1 : 1 soil-water suspension.
5. A. N. Lebedjantzev, *Soil Sci.* **18**, 419 (1924); D. A. Soulides and F. E. Allison, *ibid.* **91**, 291 (1961).
6. H. F. Birch, *Plant Soil* **10**, 9 (1958).
7. H. B. Tukey, Jr., *Bull. Torrey Bot. Club* **93**, 385 (1966).
8. J. K. McPherson and C. H. Muller, *Ecol. Monogr.* **39**, 177 (1969).
9. W. J. Peevy and A. G. Norman, *Soil Sci.* **65**, 209 (1948).
10. I thank Drs. C. H. Muller and W. H. Muller for reviews of this manuscript; I thank Dr. C. H. Muller for allowing me to use his laboratory facilities. This work was supported by NSF grant GB 14891 to C. H. Muller.

22 January 1973

Triple-Stranded Polynucleotide Helix Containing Only Purine Bases

Abstract. *The structure of the complex involving one polyadenylic acid and two polyinosinic acid chains has been determined by x-ray diffraction. The three coaxial, helical chains have conformations like conventional RNA double helices despite the absence of purine-pyrimidine pairing. Formation of hypoxanthine pairs in codon-anticodon interactions therefore requires only trivial changes in the conformation of a standard nucleotide. Evolution of the contemporary genetic code involving purine-pyrimidine complementarity from a primeval code with only adenine-hypoxanthine pairing would have been possible without major discontinuities in molecular geometry.*

We have obtained good-quality x-ray diffraction patterns (Fig. 1) from fibers of poly(I)·poly(A)·poly(I) (*1*) and have developed a detailed molecular model that corresponds closely to the diffracted intensity distribution. The molecule contains hydrogen-bonded base triplets that consist of two kinds of doubly hydrogen-bonded adenine-hypoxanthine pairs (Fig. 2a) and is of interest for a number of reasons. Its structure shows that very little change in the conformation angles of a nucleotide in a conventional RNA (purine-pyrimidine pairs) is required to accommodate such nonstandard pairs. Adenine-hypoxanthine pairs occur in codon-anticodon interactions (*2*), but the (A·I) pair (*1*) (Fig. 2a) that has the same interglycosidic link distance (10.8 Å) as a Watson-Crick pair is not, we think, as good a candidate for the "wobble" base-pairing as the (A·I) pair with the longer interglycosidic link distance (13.0 Å). It has also been suggested that adenosine phosphate and its deaminated derivative, inosine phosphate, are likely to have been the most abundant nucleotides synthesized under prebiotic conditions and that, therefore, the self-replicating precursor of the modern nucleic acids might have contained only adenine and hypoxanthine bases (*3*). Our studies suggest that nucleic acid double helices containing only the longer (A·I) pairs could well have evolved to contemporary species with purine-pyrimidine pairs with no major discontinuity of molecular geometry.

Table 1. Conformation angles in poly(I)·poly(A)·poly(I) compared with appropriate average values and estimated standard deviations (in parentheses) of nucleic acid double helices. The relevant conformation angles are defined in (*5–7, 11*) and shown in Fig. 2b. It was convenient to assume initially that poly(I_1) and poly(A) have identical conformation angles. Relaxing this requirement does not lead to an appreciably better structure.

Angle	I_1 and A (deg)	I_2 (deg)	Double helices (deg)
φ	− 66	− 61	− 69 (18)
ψ	− 73	− 57	− 69 (18)
θ	− 177	− 172	− 166 (18)
ξ	+ 63	+ 45	+ 50 (6)
σ	+ 79	+ 79	+ 83
ω	− 160	− 163	− 170 (22)
χ	+ 80	+ 83	+ 80 (9)

Although the axial rise per residue (3.3 Å) of the poly(I)·poly(A)·poly(I) helix is reminiscent of *B*-DNA, which has 3-*exo*-puckered furanose rings (*4*), the general intensity distribution of Fig. 1 is like that from all members of the *A* family of polynucleotide structures (*5*). We indeed find that all three chains are right-handed helical structures that have 3-*endo*-puckered furanose rings and very similar chain conformation angles (Table 1), none of which differs by more than 13° from the average values (*6, 7*) found in standard double helices of the *A* type.

Arnott and Hukins (*7*) and Sundaralingam (*8*) have pointed out the remarkable extent to which conformations preferred in mononucleotides are preserved in the nucleotide residues of DNA and RNA double helices. This conformational conservatism extends to this rather different triple-stranded poly(purine) nucleotide system. From another viewpoint, it means that one common nucleotide shape is sufficiently flexible, through unexceptional variations in conformation angles, to allow both complementary purine-pyrimidine pairing and purine-purine-purine triplet formation in stable helical structures.

In the model (Fig. 2), the poly(I_1) chain is antiparallel to the poly(A) chain, and the poly(I_2) is parallel (*9*). Both the antiparallel and the parallel

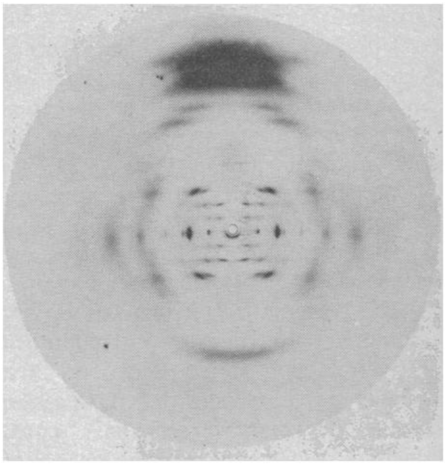

Fig. 1. Fiber diffraction pattern of the sodium salt of poly(I) · poly(A) · poly(I). The fiber was tilted 14° to the incident (Cu K_α) x-ray beam and maintained at 92 percent relative humidity during the exposure. The pattern corresponds to a helical structure with an axial rise per residue of 3.29 Å. The triple helices are packed on a hexagonal net of spacing 23.7 Å. Up-pointing and down-pointing triple helices occur randomly in the molecular sites, with the result that continuous intensity streaks accompany the sharper Bragg reflections. There is also "screw disorder" (*12*) that results in continuous diffraction in all but the center of the pattern.