

**OXYGEN AND HYDROGEN ISOTOPES IN FOSSIL INSECT CHITIN
AS PALEOENVIRONMENTAL INDICATORS**

by

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Abstract:

Isotopic analyses of the minute quantities of chitin typically available from a fossil locality is facilitated by a new method of extracting $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values from a single organic sample. This involves the pyrolysis of materials in quartz-encapsulated nickel tubes. When heated to 1050°C hydrogen diffuses through the nickel and is held in the surrounding evacuated quartz envelope. This is transferred to a mass spectrometer for analysis, then CO_2 is extracted from the nickel tube on a vacuum-separation line and analyzed for $\delta^{18}\text{O}$.

Compensation for exchangeable hydrogen is achieved through equilibration with water of known $\delta^2\text{H}$ at 0°C . In this procedure, purified chitin or cellulose is soaked in NaOH solution at about 0°C to open up the structure and make the maximum number of hydrogen atoms available for exchange. This method allows compensation for the influence of non-conservative, oxygen-bonded hydrogen in measured $\delta^2\text{H}$ values.

The utility of chitin $\delta^{18}\text{O}$ and $\delta^2\text{H}$ isotopic analyses as hydrologic and environmental indicators is demonstrated. Using modern sites from across Canada (14 sites for $\delta^{18}\text{O}$) and across North America (46 sites for $\delta^2\text{H}$), links are established between chitin isotopic content and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of environmental water, relative humidity and temperature. Chitin isotopes are also compared to cellulose $\delta^{18}\text{O}$ and $\delta^2\text{H}$ for sites where tree-feeding insects and food material were collected. These correlations are incorporated into a model with predictive capabilities which allows the determination of environmental water isotopic composition and relative humidity from chitin $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values. This is based on the Craig and Gordon model, describing steady-state evaporation from a terminal reservoir, and relates the fractionation factor between chitin and environmental water ($^{18}\alpha_{\text{chitin-environmental water}}$ and $^2\alpha_{\text{chitin-environmental water}}$) to humidity and net biochemical (α_n), equilibrium (α_e) and kinetic (α_k) fractionation factors. Evaporative enrichment of leaf water is related to α_e and α_k , which are approximated by fixed values controlled by temperature in the case of α_e and wind speed and leaf morphology in the case of α_k . The α_n is related to the biological processes which occur during synthesis of chitin and chitin precursors. For oxygen α_n is fixed, whereas α_n for hydrogen is temperature dependent, possibly

because the seasonal activity of insects in cooler climates means they are preferentially exposed to summer precipitation, resulting in relative enrichment, in an effect analogous to snowmelt bypass in lakes. Use of these expressions, along with measured humidity and temperature data results in calculated $^{18}\alpha_{\text{chitin-environmental water}}$ and $^2\alpha_{\text{chitin-environmental water}}$ values in good agreement with those observed for the North American sites analyzed.

Application of this model to a sequence of sub-till organics from central Illinois, dating from approximately 19,000 to 26,000 BP (^{14}C years), indicates a cool, moist environment, with temperatures similar to those in south-central to northern Canada today and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ environmental water values like those for southern to south-central Canada today. Insect-inferred temperature, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ environmental water and humidity generally decrease up the section, with increasing glacial proximity. Taxonomic analysis of fossil insects from this site indicates a similar trend of climatic cooling progressing up the section.

The same environmental parameters inferred from fossil cellulose in the section are similar in magnitude and trend, with the exception of humidity, which is lower than that inferred from chitin and displays a general increase up the section. The wetter chitin-inferred environment is attributed to the more protected forest-floor conditions in which most insects live. The canopy of trees would retain humidity and provide protection from wind, resulting in a decrease in evaporation.

A serendipitous result of these differing inferred-humidity values is that chitin and cellulose isotopic values can be combined to constrain an evaporation line on a $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ plot, which can be extrapolated to the global meteoric water line ($\delta^2\text{H} = 8\delta^{18}\text{O} + 10$) to yield inferred environmental water $\delta^{18}\text{O}$ and $\delta^2\text{H}$.

To Heather, Will and Kate.

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Chapter 1:

Introduction:

The thrust of the research in this thesis is the development of isotopic analysis of fossil insect chitin as a paleoenvironmental tool. However, in order to provide a proper framework for this study, a brief discussion of isotope hydrology in general and isotope paleoenvironmental investigations using cellulose as an archive are in order.

It has long been recognized (Dansgaard, 1964) that the isotopic composition of precipitation varies in a consistent pattern worldwide in a way which reflects, in a general sense, the latitudinal climatic gradient. In short, precipitation is isotopically heavier at low latitudes where climate is generally warm and becomes progressively lighter in cooler climates northwards. Rozanski *et al.* (1993) noted that two-thirds of global oceanic evaporation originates between the latitudes 30°N and 30°S. Rainout lightens the isotopic content of this air-mass moisture as it progresses north and south. Dansgaard (1964) found spatial isotope-temperature gradients of 0.69‰/°C for $\delta^{18}\text{O}$ and 5.6‰/°C for $\delta^2\text{H}$ in weighted mean annual precipitation, which reflect the global meteoric water line slope of 8.0 ($\delta^2\text{H} = 8\delta^{18}\text{O} + 10$) (Craig, 1961). Rozanski *et al.* (1992,1993) found about a 0.6‰/°C isotope-temperature gradient for $\delta^{18}\text{O}$ in precipitation for mid and high-latitude stations in the International Atomic Energy Agency/World Meteorological Organization (IAEA/WMO) network. Higher gradients, from 0.67‰ to 0.90‰/°C, were encountered in northern and southern polar latitudes (Rozanski *et al.*, 1993).

The rainout of isotopically-heavier moisture from an air mass, resulting in a progressive depletion of the remaining water vapour, can be modeled as a Rayleigh-type distillation process (Rozanski *et al.*, 1992, 1993; Jouzel *et al.*, 1999). In Rayleigh-type distillation liquid water is condensed from an air mass and is immediately removed through precipitation. The isotopic content of the condensate is a function of the original composition of the water vapour and a temperature-dependent fractionation factor. The result is continuous removal of heavier water and isotopic depletion of the water-vapour

reservoir, which leads to progressively-lighter condensate. These processes are described by the equations (Rayleigh, 1896):

$$R_V/R_{V0} = f^{(\alpha - 1)}$$

$$R_L/R_{V0} = \alpha f^{(\alpha - 1)}$$

Where R_{V0} is the isotopic ratio (i.e. $^{18}\text{O}/^{16}\text{O}$) in the initial vapour reservoir, f is the residual fraction of the original vapour, R_V is the instantaneous isotopic ratio of the remaining vapour, R_L is the instantaneous isotopic ratio of the liquid leaving the vapour and α is the fractionation factor R_L/R_V .

Over this general pattern are superimposed the effects of atmospheric circulation and local climatic influences and the proximity to significant geographic features, such as mountains and lakes. Stated in another way, on a large scale, the worldwide distribution of isotopes in precipitation is a simple hydrologic system, governed by Rayleigh-type processes which are modified by climatological and geographic variables.

Armed with this knowledge, it is clear that if archives of paleo-precipitation can be tapped, information they yield can be combined with modern observations of the relationship between isotopes in precipitation and climate to provide a window on climates in the past. However, while this concept is simply stated, it is not so simple in execution. Ideally, an archive would preserve the isotopic content of precipitation with little modification. Ice, preserved in ice sheets and glaciers is such an archive, with the added advantage of good chronological control provided by annual layers (Rozanski *et al.*, 1997; Jouzel *et al.*, 1999). The disadvantage of ice is that long-term records are only preserved in high-latitude continental ice sheets, such as those in Greenland, Antarctica and northern Canada, and in high-altitude glaciers at lower latitudes. Groundwater provides another archive of paleo-precipitation (Rozanski *et al.*, 1997), although it is commonly more limited in time span than glacial ice and lacks the chronological control.

1.1: Plant archives:

Cellulose ((C₆H₁₀O₅)_x), the basic structural component of plants, has received considerable attention as an archive of paleo-precipitation, based on the premise that environmental water is the source of hydrogen and oxygen in cellulose (Smith and Epstein, 1970; Epstein *et al.* 1976; and Epstein *et al.* 1977). The conceptual model behind this is illustrated in Figure 1.1. In it, precipitation is taken up by plants as groundwater and has its isotopic composition modified by evaporative and biological processes before it is finally incorporated in cellulose precursors and cellulose.

Cellulose forms part of the cell walls of photosynthesizing plants. It is a linear condensation polymer consisting of a series of glucose molecules bonded together in the form of a flat ribbon. Wood contains between 40 and 55 per cent cellulose (Nevell and Zeronian, 1985).

While some pioneering work (Schiegl, 1974) used D/H ratios (the ratio of deuterium to hydrogen) in whole-wood samples as climatic indicators, Epstein *et al.* (1976) argued that the chemistry of plants is too complex for this approach to give accurate results. They said analysis of the D/H in whole-plant material could be affected by varying isotopic compositions of different substances within the plants. Therefore, isotopic differences ascribed to climatic influences could be due to differences in plant chemistry. The solution to this problem lay in analyzing only the cellulose, thus eliminating the difficulties caused by differing isotopic compositions of various components (Epstein *et al.*, 1976). In the cellulose molecule hydrogen is bound to both oxygen and carbon. The hydroxyl hydrogen is exchangeable and can, therefore, be isotopically altered after cellulose formation, leading to variations in $\delta^2\text{H}$ unrelated to environmental water at the time of cellulose synthesis. This difficulty can be circumvented by removing the exchangeable hydrogen, using a nitration procedure, and performing the isotopic analysis on cellulose nitrate: C₆H₇O₂(NO₃)₃ (Epstein *et al.*, 1976).

The greater accuracy inherent in cellulose nitrate $\delta^2\text{H}$ analysis was illustrated by the determination of $\delta^2\text{H}$ for both raw wood and cellulose nitrate from two bristlecone pines from California (Epstein *et al.*, 1976; Epstein and Yapp, 1976). For six time-equivalent samples from the two trees, $\delta^2\text{H}$ values varied by +10 to -30‰ for raw wood but were within

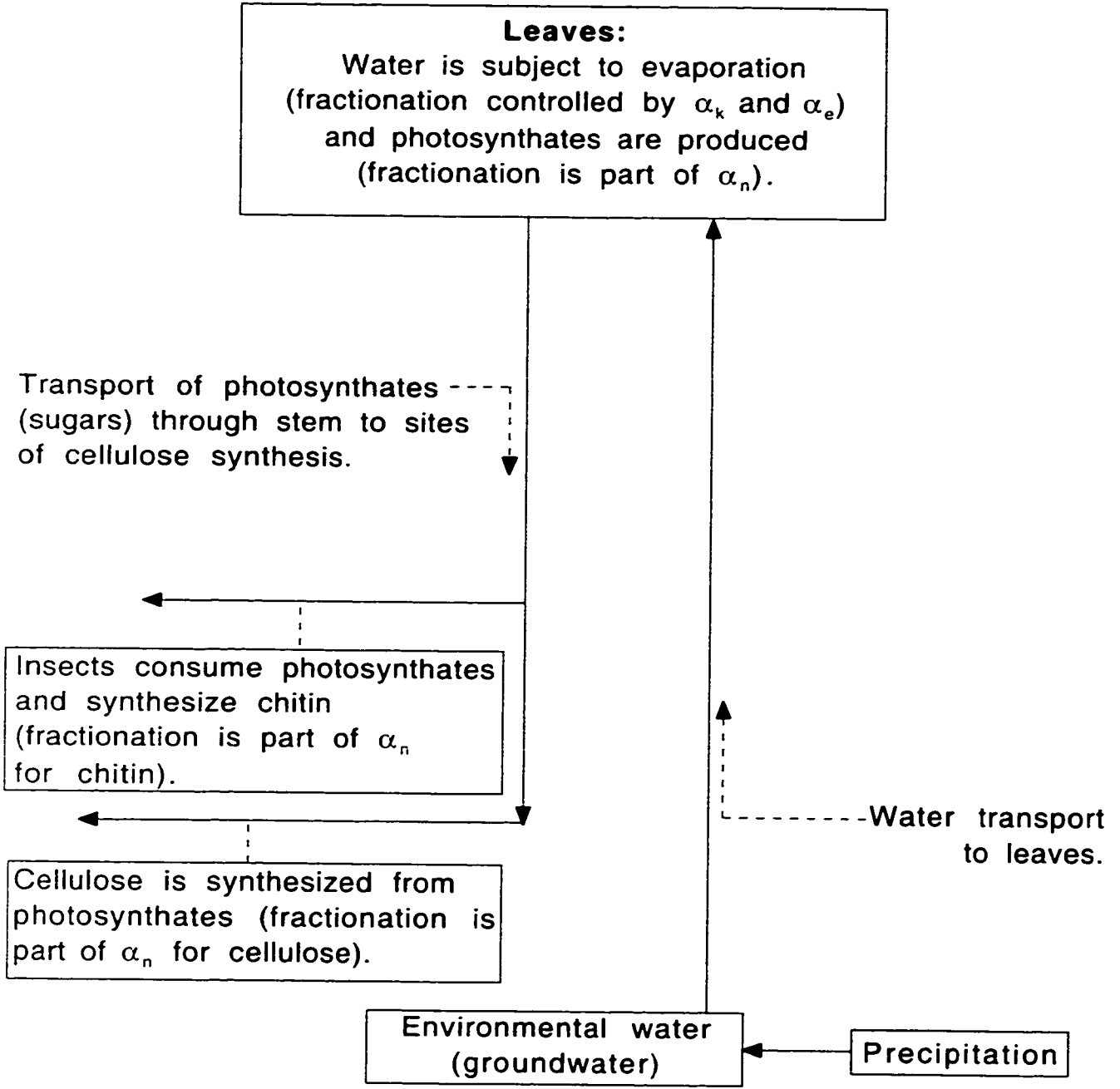


Figure 1.1: Schematic representation of the conceptual model underlying chitin and cellulose paleohydrological research. It outlines how environmental water is modified by fractionating processes (evaporative and biological) before its isotopic composition is incorporated into chitin and cellulose.

experimental precision of 2‰ for all but one pair of cellulose-nitrate samples. Determinations of cellulose-nitrate $\delta^2\text{H}$ in a variety of plants and their associated environmental water established that there is a strong correlation between the two values (Epstein *et al.*, 1976).

With the superiority of cellulose-nitrate $\delta^2\text{H}$ analysis established, Epstein and Yapp (1976) proceeded to determine the climatic relevance of $\delta^2\text{H}$ measurements. They showed that there was a linear relationship between cellulose-nitrate $\delta^2\text{H}$ and the $\delta^2\text{H}$ of water associated with the plants. The slope of this relationship was close to one (0.97), demonstrating that the isotopic content of source water was the primary factor controlling the isotopic composition of the cellulose nitrate. An analysis of a Scots pine from Scotland yielded a cellulose $\delta^2\text{H}$ record for the period 1841 to 1970. The 40-year running average of decadal means showed a good correlation with the same average of October to March temperatures for Edinburgh over the period covered by the wood sample. When the 40-year running averages for the Scots pine and bristlecone pine were plotted against each other, the result was a linear relationship which suggested the $\delta^2\text{H}$ records from both trees were reflecting long-term climatic trends in the northern hemisphere. This temperature relationship was presumably inherited from variations in precipitation $\delta^2\text{H}$.

In another study (Yapp and Epstein, 1982a) the $\delta^2\text{H}$ was measured for cellulose from 25 trees and for precipitation from 11 International Atomic Energy Agency stations across North America. Plotting the $\delta^2\text{H}$ versus average annual temperature for the sites yielded a $\delta^2\text{H}$ gradient of 5.6‰/°C for precipitation and 5.8‰/°C for cellulose nitrate: demonstrating a good correlation between the two $\delta^2\text{H}$ measurements. Furthermore, the cellulose $\delta^2\text{H}$ values showed a decrease from coast to interior and south to north: the same pattern demonstrated for $\delta^2\text{H}$ from meteoric waters.

Further refinement of our understanding of the relationship between cellulose $\delta^2\text{H}$ and climate was achieved through examination of the effects of evapotranspiration on isotopic fractionation in plants (Yapp and Epstein, 1982b). In that study the authors plotted cellulose $\delta^2\text{H}$ versus environmental water $\delta^2\text{H}$ for a variety of plants from a selection of geographic locations. While the result was a good correlation between

the two variables, they suggested that scatter among the points indicated that something other than meteoric water $\delta^2\text{H}$ was affecting the cellulose $\delta^2\text{H}$.

Evapotranspiration results in a considerable volume of water leaving plants through the leaves and leads to an increase in the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of leaf water, since water composed of lighter isotopes evaporates more readily (Yapp and Epstein, 1982b). It follows from this that relative humidity would have an effect on evapotranspirative enrichment because there is greater evaporation, and therefore greater enrichment, when humidity is lower. The relationship between humidity and fractionation was modeled by the equation:

$$\frac{1000 + \delta^2\text{H}_{\text{cellulose}}}{1000 + \delta^2\text{H}_{\text{environmental water}}} = {}^2\alpha_n {}^2\alpha_e {}^2\alpha_k - {}^2\alpha_n ({}^2\alpha_e {}^2\alpha_k - 1)h = {}^2\alpha_{\text{cellulose-water}}$$

Where ${}^2\alpha_n$ is the net biochemical fractionation factor for hydrogen during cellulose synthesis from plant water; ${}^2\alpha_e$ and ${}^2\alpha_k$ are the equilibrium and kinetic fractionation factors for hydrogen, between the liquid and vapour phases of water; h is the relative humidity, expressed as a decimal fraction; and ${}^2\alpha_{\text{cellulose-water}}$ is the total fractionation factor between cellulose and environmental water. This relationship is based on the Craig and Gordon model (Craig and Gordon, 1965), describing steady-state evaporation from a terminal reservoir. By plotting the total fractionation factor against growing season average relative humidities Yapp and Epstein (1982b) were able to demonstrate a roughly linear correlation between the two factors, showing that relative humidity has an influence on $\delta^2\text{H}$ of carbon-bound cellulose hydrogen.

The effects of evapotranspirative enrichment, related to relative humidity, also have a profound influence on oxygen isotopes in plants. Epstein *et al.* (1977) found that water extracted from leaves of terrestrial plants fell along a line with slope 2.5 on a $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ plot as compared to a slope of 8.0 for the global meteoric water line (Craig, 1961). This reduction in slope would be the result expected if evapotranspiration were enriching the $\delta^{18}\text{O}$ content of plant water to a greater extent than $\delta^2\text{H}$. Water from a pond-lily leaf plotted in between the slope 8.0 and slope 2.5 lines: an anticipated result since that plant

grows under more humid conditions at the water's surface and would be subject to less evapotranspiration. The effects of humidity could also be seen in measured values of cellulose $\delta^{18}\text{O}$ in 23 terrestrial plants, which were generally more positive than a theoretical curve which modeled $\delta^{18}\text{O}$, as a function of $\delta^2\text{H}$ of environmental water, disregarding evapotranspiration (Epstein *et al.*, 1977).

These authors also found that cellulose $\delta^{18}\text{O}$ in trees from Florida and from the Yukon varied by only 9.0 per mil, as compared to a 178-per-mil variation between the $\delta^2\text{H}$ values in the same trees. Furthermore, when aquatic plants and terrestrial ones had the same $\delta^2\text{H}$, the $\delta^{18}\text{O}$ of the land plants was found to vary by 4.0 to 16 per mil. They concluded that $\delta^{18}\text{O}$ is relatively insensitive to climatic temperature variations, although it could be used, along with $\delta^2\text{H}$, to analyze the humidity conditions under which plants grew.

Burk and Stuiver (1981) drew a different conclusion in their study of cellulose $\delta^{18}\text{O}$ from trees near the Pacific coast of the United States, from California to Alaska, and from Mount Rainier, Washington. They found that the cellulose $\delta^{18}\text{O}$ in coastal trees varied at a rate of $0.41\text{‰}/^\circ\text{C}$, which compared favorably with the spatial $\delta^{18}\text{O}$ -temperature gradient for precipitation along the Pacific coast of $0.43\text{‰}/^\circ\text{C}$. Using the $0.41\text{‰}/^\circ\text{C}$ figure and the $\delta^{18}\text{O}$ of tree cellulose from Mount Rainier, they calculated a lapse rate of $5.2 \pm 0.5^\circ\text{C}$ per 1,000 m, which is close to the measured lapse rate of 5.0°C per 1,000 m.

These results indicated a stronger relationship between temperature and cellulose $\delta^{18}\text{O}$ than Epstein *et al.* (1977) found. However, Burk and Stuiver (1981) selected their tree sites to minimize variations in humidity. In fact, humidity at their sites was a relatively constant $67 \pm 6\%$, meaning that one of the principal factors, evapotranspirative enrichment, affecting cellulose $\delta^{18}\text{O}$ in the earlier study likely did not produce much variation in the values for coastal trees.

The relationship between evapotranspirative enrichment of leaf water and atmospheric moisture content suggests that $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements could be used to calculate relative humidity. Burke and Stuiver (1981) derived an expression which related $\delta^{18}\text{O}$ of cellulose, $\delta^{18}\text{O}$ of source water and humidity. They also considered such factors as the

oxygen isotopic fractionation between atmospheric CO₂ and water vapor, temperature, and kinetic and equilibrium fractionation.

This model was necessarily complex because of the many factors which had to be considered. Edwards *et al.* (1985) found that a linear relationship exists between relative humidity and enrichment of cellulose in δ¹⁸O, suggesting that many of the factors in Burke and Stuiver's (1981) model need not be considered. Edwards and Fritz (1986) determined that this linear relationship could be described by the equation:

$$\frac{1000 + \delta^{18}\text{O}_{\text{cellulose}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}} = {}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k - {}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)h = {}^{18}\alpha_{\text{cellulose-water}}$$

Where ¹⁸α are the fractionation factors affecting oxygen. This is the same form as the expression derived by Yapp and Epstein (1982b) for the relationship between ²α_{cellulose-water} and relative humidity. If the two equations were combined and the equation for the global meteoric water line (Craig, 1961) δ²H_{environmental water} = 8 δ¹⁸O_{environmental water} + 10 was used to replace δ²H_{environmental water} in the above expressions, δ¹⁸O_{environmental water} and humidity values could be calculated from any pair of cellulose δ¹⁸O and δ²H measurements.

Using these equations, Edwards and Fritz (1986) calculated humidities and δ¹⁸O_{environmental water} for a variety of sites in Canada and compared them to measured values for the same locations. The results showed good agreement between measured and calculated values. This model was further refined by Buhay *et al.* (1996) with better characterization of kinetic fractionation factors affecting leaf water during evapotranspiration.

The model cannot be applied uncritically, however: trees from environments with short groundwater residence times may reflect short-term fluctuations in δ¹⁸O and δ²H rather than the integrated signal characteristic of well-mixed environmental water (Edwards, 1993). Evaporation of environmental water prior to absorption by the tree could lead to enrichment which moves the isotopic composition of source water off the meteoric water line (cf. Yapp and Epstein, 1982b; Edwards, 1993). Inconsistent growth rates could lead to inferred humidity values

which do not represent an integrated annual value, but are more indicative of periods of rapid growth. This effect would be most pronounced in extreme climates where conditions limit growth to certain periods of the day (Edwards, 1993). Even in temperate climates, where conditions favor uniform growth, early and late wood have been found to have different isotopic signatures (Epstein and Yapp, 1976).

A more fundamental issue is whether the models relating humidity and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ actually reflect the processes going on in the plant. Based on experiments using potatoes, DeNiro and Cooper (1989) concluded that the oxygen isotopic composition of cellulose is affected by unenriched water contained in the roots and shoots of the plant, so cellulose $\delta^{18}\text{O}$ values may not be sensitive to humidity. These authors did not test whether this holds true for $\delta^2\text{H}$, but said they expect it is the case. Edwards (1990) argued that the weight of empirical evidence (Yapp and Epstein, 1982b; Burke and Stuiver, 1981; Edwards *et al.*, 1985; Edwards and Fritz, 1986) suggested that these models do accurately reflect processes occurring within the plant. He went on to state that DeNiro and Cooper's (1989) findings may indicate that some other process is mimicking the isotopic effects which would result from evapotranspirative enrichment of leaf water.

Further work has continued to suggest re-equilibration of cellulose precursors with water, prior to cellulose synthesis without providing an explanation for the agreement between the model and observations: Yakir and DeNiro (1990) grew *Lemna* plants under controlled conditions and found that 70% of oxygen and carbon bound hydrogen were exchanged with water prior to cellulose synthesis. Yakir (1992) stated that the location of metabolic water with respect to the site of evaporation may vary, citing results in Yakir *et al.* (1990) which showed that leaf water cellulose $\delta^{18}\text{O}$ did not respond to changes in humidity. Dawson and Ehleringer (1993) speculated that their observed correlation between tree-ring cellulose nitrate $\delta^2\text{H}$ and environmental water $\delta^2\text{H}$ may indicate that cellulose synthesis is influenced by the presence of unfractionated water in the tree trunk.

White *et al.* (1994) noted that relative humidity affected cellulose $\delta^2\text{H}$ values, but suggested that this effect is damped by humidity-related changes in the isotopic content of atmospheric water vapour. Saurer *et*

al. (1997) and Anderson *et al.* (1998) found a relationship between tree-stem cellulose $\delta^{18}\text{O}$ and relative humidity. Both papers concluded, however, that the signal was damped to a certain extent, possibly through isotopic exchange between cellulose precursors and unenriched stem water or compartmentalization of leaf water (Yakir *et al.*, 1994), causing some leaf water to be inaccessible to evaporation. The net effect of this damping (termed the *f* factor) was that 40 to 70% of the leaf water was not subject to evaporative enrichment. Buhay *et al.* (1996) stated that *f*-factor damping could be resolved through selection of kinetic fractionation factors which vary with leaf morphology. Those authors go on to state that evaporatively-enriched plant water may act as the transport medium for cellulose precursors, enroute to sites of cellulose synthesis, thus preventing exchange with unenriched water.

1.2: Insect archives:

Quaternary paleoentomological studies have been in progress since the latter half of the 19th century (Morgan and Morgan, 1980; Elias, 1993). Over the last 25 years fossil insects, particularly beetles, have gained prominence as paleoenvironmental indicators (Coope, 1970; Morgan, 1973; Morgan and Morgan, 1979; Schwert *et al.*, 1985; Motz, 1990 and Motz and Morgan, 1997). These efforts have used fossil insects as proxy indicators of climate, in much the same way as pollen, macroscopic plant remains and other fossils are used in paleoenvironmental reconstructions.

The basic structural component of the insect exoskeleton is chitin: a polysaccharide with the generalized formula $(\text{C}_8 \text{H}_{13} \text{O}_5 \text{N})_x$. It is resistant to degradation, resulting in excellent preservation under conditions of fossilization (Schimmelman *et al.*, 1986; Stankiewicz *et al.*, 1997), and facilitating separation from other materials prior to analysis. Chitin is chemically and structurally similar to cellulose. Because of this similarity, and the fact that the beginning of the food chain which leads to chitin is plant material (Figure 1.1), it is to be expected that fossil insects can provide isotope paleoenvironmental information similar to that yielded by cellulose.

Investigations into the application of isotope paleoclimatological techniques to fossil insect materials began with the work of Miller

(1984) (see also Miller *et al.*, 1988). He established a link between insect chitin carbon and hydrogen isotopic composition and diet in laboratory tests and, in a general way, in the natural environment. He also demonstrated a relationship between chitin hydrogen isotopic composition and meteoric water isotopic composition and temperature, under modern conditions in North America. Schimmelmann *et al.* (1993) showed that this relationship may be useful as a paleoclimatic tool, although that study was limited to only five samples from a late-glacial sequence in Nova Scotia and, while providing summer-temperature estimates, made no attempt to infer $\delta^2\text{H}_{\text{environmental water}}$ or other environmental parameters. Nevertheless, the authors did find that the climatic trends indicated by insect chitin hydrogen isotopes were similar to temperature trends seen in some European late-glacial sequences and agreed with palynological climatic evidence from the same period in eastern Canada.

This contradicts the results of Schimmelmann and DeNiro (1986a) who analyzed a variety of terrestrial, aquatic and marine arthropods and found temperature-related effects on hydrogen isotopes of chitin were obscured by the natural isotopic noise levels. They also found only a weak correlation between chitin isotopic composition and that of the ambient water. Oxygen isotopes in chitin were also studied by Schimmelmann and DeNiro (1986a), yielding results similar to those for hydrogen isotopes. No attempt was made with either element to compensate for effects related to relative humidity or seasonality of the arthropod's lifestyle. Furthermore, a mechanistic model to account for the observed effects was not proposed. Their data show that $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in chitin were significantly enriched over environmental water. While some of this effect may be due to biological fractionation inherent in chitin synthesis, part of it could result from evaporative enrichment related to humidity. However, without detailed humidity or other environmental information it is difficult to decode the isotopic signal displayed by the chitin.

1.3: Goals of paleoenvironmental research:

The ultimate purpose of paleoenvironmental analysis using any of the available tools is two-fold:

- i) To elucidate past climates. This goal is fulfilled by any paleoenvironmental data, whether qualitative or quantitative, provided it can be located within temporal and geographic space.
- ii) To validate or test numerical climatological models. Fulfillment of this goal is predicated upon the availability of quantitative data, such as that inferred from isotopic analysis of precipitation archives, which can be incorporated in these models.

Both of these goals can be achieved by paleoentomological research. It is unusual in its ability to provide qualitative climatic data through traditional taxonomic analysis of fossil insect assemblages and has the potential to provide quantitative information through isotopic analysis. Furthermore, chitin isotopes may provide different information from that available from cellulose analysis because of the different conditions under which plants and insects exist. If chitin isotopic analysis can be applied to individual species or genera, then detailed information relating to microenvironments or trophic level could become available.

1.4: Purpose of this thesis:

Using past isotope paleoclimatic investigations based on plant cellulose and arthropod chitin as starting points, the primary goal of my research was: To investigate the use of hydrogen and oxygen isotopes in insect chitin as paleoenvironmental indicators. This overall theme can be divided into several subthemes:

- i) To develop new laboratory methods for doing isotopic research on insect chitin in order to make the technique more applicable to fossil material, which is usually available only in extremely small quantities.
- ii) To further elucidate the relationship between the isotopic composition of an insect's food source and its chitin in the natural environment.

iii) To more fully explore the way in which isotopic variations in an insect's integument reflect its environment: specifically temperature and hydrology.

iv) To use i, ii and iii to study paleoenvironments using fossil insects.

1.5: Notation:

All isotopic measurements are expressed in conventional isotopic notation:

$$\delta^{18}\text{O}_{\text{sample}} (\text{‰}) = \frac{(^{18}\text{O}_{\text{sample}}/^{16}\text{O}_{\text{sample}}) - (^{18}\text{O}_{\text{SMOW}}/^{16}\text{O}_{\text{SMOW}})}{(^{18}\text{O}_{\text{SMOW}}/^{16}\text{O}_{\text{SMOW}})} \times 1,000$$

$$\delta^2\text{H}_{\text{sample}} (\text{‰}) = \frac{(^2\text{H}_{\text{sample}}/^1\text{H}_{\text{sample}}) - (^2\text{H}_{\text{SMOW}}/^1\text{H}_{\text{SMOW}})}{(^2\text{H}_{\text{SMOW}}/^1\text{H}_{\text{SMOW}})} \times 1,000$$

The standard used for both oxygen and hydrogen is Vienna Standard Mean Ocean Water (VSMOW) (Coplen, 1996), normalized such that Standard Light Antarctic Precipitation (SLAP) = -55.5‰ and -428‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ respectively.

Chapter 2:

Procedures for analyzing hydrogen and oxygen isotopes:

Isotope analysis of fossil insects poses two special problems:

- 1) Sample sizes are very small.
- 2) Exchangeable hydrogen must be eliminated or compensated for.

2.1: Hydrogen and oxygen isotopic analysis of organic materials using nickel pyrolysis:

In order to deal with the restricted amounts of material a technique was developed which allows both hydrogen and oxygen analyses from a single small sample (Motz *et al.*, 1997).

Modifications to a nickel-pyrolysis technique have enhanced the efficiency of $\delta^{18}\text{O}$ analyses on micro-water and organic samples (Edwards, *et al.*, 1994). The hydrogen that diffuses through the nickel lattice during pyrolysis also provides the opportunity to evaluate the hydrogen-isotopic compositions of these types of samples (as originally suggested by Thompson and Gray (1977)).

Current methodology for analyzing water samples involves production of H_2 gas by reduction with zinc, for a precision of $\pm 2\%$. For organic samples, the material is oxidized in-vacuo, by heating with cupric oxide, to produce water, which is then reduced by zinc, for a total precision on the order of $\pm 3-4\%$ for individual determinations.

This section describes the accuracy and precision of $\delta^2\text{H}$ analyses of hydrogen collected during the nickel pyrolysis of water and polyethylene of known hydrogen isotopic composition, as well as the precision obtained from analyses of organic samples, such as cellulose, insect chitin and peptide. For some samples the CO_2 pyrolysis products were collected and analyzed for their oxygen-isotope compositions to evaluate the feasibility of measuring isotopes of both elements from the same sample.

2.2: Method:

The nickel pyrolysis bombs used in these experiments were similar to those described in Edwards *et al.* (1994). They consisted of tubes machined from nickel-200 rod, 9 cm long, 10 mm outside diameter and 6 mm inside diameter, with one closed end. Samples were introduced through the open end, then the bomb was flushed with argon and sealed using CAJON VCR-style fittings and a 5 mm silver-plated nickel blind gasket. Containment of hydrogen diffusing through the nickel bomb walls was accomplished by inserting the bombs into quartz tubes (25 mm outside diameter, 22 mm inside diameter and 210 mm long) which were closed at one end. The open ends of the tubes were attached, through CAJON fittings, to valves, which allowed evacuation of the apparatus and removal of hydrogen following pyrolysis (Motz *et al.*, 1997).

This technique was first evaluated and calibrated using water standards for $\delta^2\text{H}$, and water and cellulose standards for $\delta^{18}\text{O}$. It was then applied to IAEA-standards C3 cellulose and CH-7 polyethylene, chitin from the ambrosia beetle (Coleoptera, Scolytidae) *Tripodendron lineatum*, bound water in biotite mica (NBS-30 standard) and peptide to determine whether consistent results could be obtained from these materials.

Using a glass syringe, water samples (10 μl) encompassing a range of isotopic compositions were injected into pyrolysis bombs containing graphite (10 mg). CH-7 samples (about 9 mg) were combined with cupric oxide (about 163 mg) and carbon (about 5 mg). The cupric oxide apparently oxidized the polyethylene, while the carbon reduced any water which formed. Biotite (about 40 mg) was sealed in bombs with carbon (about 13 mg). Cellulose (about 25 mg), chitin (about 20 mg) and peptide (about 9 mg) samples were inserted into bombs and sealed in an argon environment.

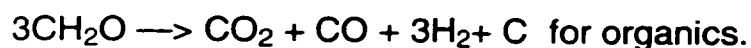
After loading, each bomb was inserted into a quartz tube, which was sealed and evacuated to 10^{-3} torr. The lower portion of the quartz tube, with the nickel pyrolysis bomb, was heated (at 1050°C) in an electrical-resistance oven, during which time hydrogen diffusing through the nickel was retained in the quartz tubes.

Hydrogen was transferred directly from the quartz tubes to a mass spectrometer for analysis within a few hours of pyrolysis. In the cases

of water and cellulose samples, gases were removed from the bombs and CO₂ was cryogenically separated for δ¹⁸O analysis.

2.3: Discussion:

The δ²H and δ¹⁸O values for water and IAEA CH-7 samples at reaction times between 20 and 80 minutes, in comparison with their known isotopic values, are provided in Table 2.1. True values for standards were determined using zinc reduction for δ²H and CO₂ equilibration for δ¹⁸O. Corrected δ²H values were calculated using the correction equation derived from the regression of Experiment 1 in Figure 2.2. Corrected δ¹⁸O values were obtained from the standard University of Waterloo Stable Isotope Laboratory equation $\delta^{18}\text{O}_{\text{corrected}} = 1.203 \delta^{18}\text{O}_{\text{RAW}} + 14.528$. The development of zero-departure (from known values) plateaus for both hydrogen and oxygen isotopic values from water and CH-7 samples between 65 and 80 minutes of reaction time, shown in Figure 2.1a—e, suggests a completed pyrolysis reaction:



STANDARD (true δ value)	PYROLYSIS RESULTS	HEATING TIME IN MINUTES				
		20	35	50	65	80
Water:						
$\delta^2\text{H}_{\text{SMOW}} = 26.81$	$\delta^2\text{H}_{\text{SMOW}}$	-175.82	-21.76	2.83	21.29	15.6
		-149.81		2.99	16.43	
				15.16		
	average	-163.92	-21.76	2.91	17.63	15.60
	stand. dev.	13.14		0.11	3.24	
	corrected	-175.73	-16.65	10.95	27.42	25.15
	departure	-202.54	-43.46	-15.86	0.61	-1.66
$\delta^{18}\text{O}_{\text{SMOW}} = -10.75$	$\delta^{18}\text{O}_{\text{RAW}}$	-9.29	-17.98	-18.79	-20.39	-19.84
				-21.39	-22.27	
	average	-9.29	-17.98	-20.09	-21.33	-19.84
	corrected	3.35	-7.10	-9.64	-11.13	-9.34
	departure	14.1	3.65	1.11	-0.38	1.41
Water:						
$\delta^2\text{H}_{\text{SMOW}} = -38.88$	$\delta^2\text{H}_{\text{SMOW}}$	-156.31	-66.57	-41.52	-35.67	-38.63
				-41.32	-38.28	
				-53.76		
	average	-156.31	-66.57	-45.53	-36.98	-38.63
	stand. dev.			7.13	1.85	
	corrected	-167.22	-66.80	-43.25	-33.69	-35.53
	departure	-128.34	-27.92	-4.37	5.19	3.35
$\delta^{18}\text{O}_{\text{SMOW}} = -10.05$	$\delta^{18}\text{O}_{\text{RAW}}$	-17.20	-17.28	-18.58	-18.90	
				-18.76	-21.79	
	average	-17.20	-17.28	-18.67	-20.35	
	corrected	-6.16	-6.26	-7.93	-9.95	
	departure	3.89	3.79	2.12	0.1	
Water:						
$\delta^2\text{H}_{\text{SMOW}} = -115.3$	$\delta^2\text{H}_{\text{SMOW}}$	-259.04	-142.65	-113.4	-111.07	-111.37
				-112.03	-118.53	
	average	-259.04	-142.65	-112.72	-114.80	-111.37
	stand. dev.			0.97	5.28	
	corrected	-282.17	-151.93	-118.44	-120.77	-116.93
	departure	-166.83	-36.59	-3.1	-5.43	-1.59
	$\delta^{18}\text{O}_{\text{SMOW}} = -11.13$	$\delta^{18}\text{O}_{\text{RAW}}$	-13.23	-19.11	-20.99	-20.09
					-22.76	
average		-13.23	-19.11	-20.99	-21.43	-21.47
corrected		-1.39	-8.46	-10.72	-11.25	-11.30
departure		9.74	2.67	0.41	-0.12	-0.17

Table 2.1: Continued on next page.

STANDARD (true δ value)	PYROLYSIS RESULTS	HEATING TIME IN MINUTES					
		20	35	50	65	80	
CH-7:							
$\delta^2\text{H}_{\text{SMOW}} = -100.3$	$\delta^2\text{H}_{\text{SMOW}}$	-230.84	-131.84	-99.06	-99.97	-97.01	
	corrected	-250.61	-139.83	-103.15	-104.17	-100.86	
	departure	-150.31	-39.53	-2.85	-3.87	-0.56	
		20	30	35	50	60-70	80
Water:							
$\delta^2\text{H}_{\text{SMOW}} = -198$	$\delta^2\text{H}_{\text{SMOW}}$	-255.86	-233.7	-217.87	-182.09	-183.1	-175.89
					-191.83	-182.59	-181.78
						-183.5	
	average	-255.86	-233.70	-217.87	-186.96	-183.06	-178.84
	stand. dev.				6.89	0.46	4.16
	corrected	-278.61	-253.82	-236.10	-201.51	-197.15	-192.43
departure	-80.61	-55.82	-38.1	-3.51	0.85	5.57	
$\delta^{18}\text{O}_{\text{SMOW}} = -26.3$	$\delta^{18}\text{O}_{\text{RAW}}$	-26.75	-23.60	-31.61	-34.52	-29.80	-33.53
						-35.56	
	average	-26.75	-23.60	-31.61	-34.52	-32.68	-33.53
	corrected	-17.65	-13.86	-23.50	-27.00	-24.79	-25.81
	departure	8.65	12.44	2.8	-0.7	1.51	0.49

Table 2.1: Hydrogen and oxygen isotopic analyses of water and polyethylene-foil standards. $\delta^{18}\text{O}_{\text{RAW}}$ is with respect to SMOW. All values are in parts per mil.

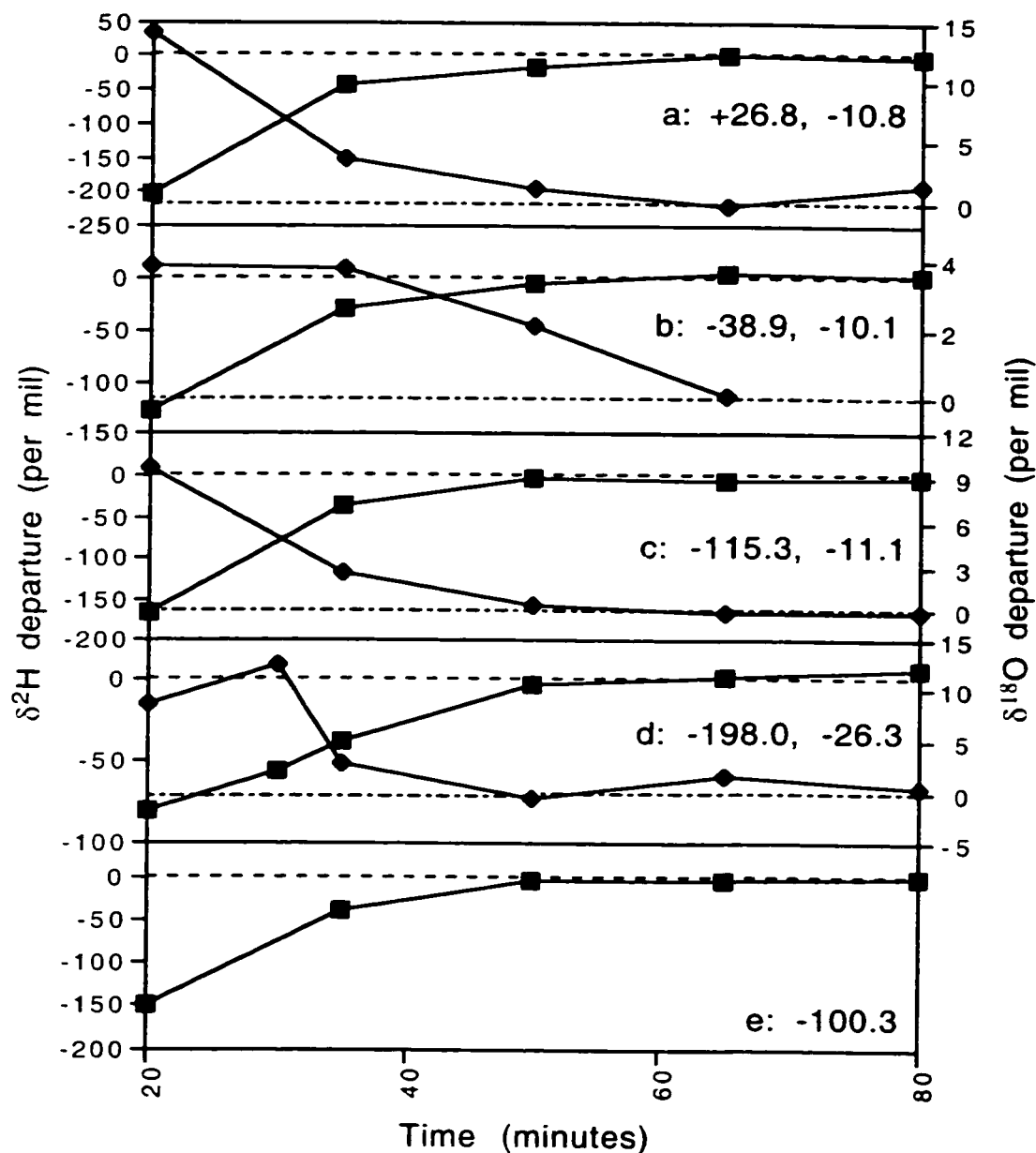


Figure 2.1: Effects of pyrolysis times on departures from known $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of water (a to d) and polyethylene foil (e) standards. Squares are $\delta^2\text{H}$ and diamonds are $\delta^{18}\text{O}$. Known values for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ ($\delta^2\text{H}$ only for e) respectively are shown. Zero departure plateaus for $\delta^2\text{H}$ are broken lines while those for $\delta^{18}\text{O}$ are dot-dash lines. All analyses were performed in Experiment 1-style bombs.

The $\delta^2\text{H}$ values of the samples subjected to shorter reaction times are consistently depleted with respect to their known values (negative departures in Figure 2.1a—e). Depleted $\delta^2\text{H}$ values early in the reaction are probably a result of isotopic fractionation arising from the preferential breaking of $^1\text{H-O}$ bonds over $^2\text{H-O}$ bonds in water and $^1\text{H-C}$ bonds over $^2\text{H-C}$ in polyethylene foil and, possibly, rapid diffusion of ^1H through the nickel lattice. Almost-quantitative (see discussion of correction lines below) recovery of the hydrogen and deuterium is indicated by the development of $\delta^2\text{H}$ zero-departure plateaus for each of the water and CH-7 samples. Enriched $\delta^{18}\text{O}$ values of samples subjected to shorter reaction times probably reflect isotopic non-equilibrium between CO and CO_2 early in the pyrolysis reaction, as suggested by Edwards *et al.* (1994).

Figure 2.2 shows $\delta^2\text{H}$ correction lines, based on water standards reacted for 65 minutes, for three different styles of pyrolysis bombs. The data for Experiment 1 were obtained with the bombs used for the analyses reported in Table 2.1 and Figure 2.1 (Motz *et al.*, 1997). Results for Experiment 2 (Appendix 1, Table A) were obtained with a new bomb, which is approximately 2 cm longer and used stainless steel rather than nickel fittings (Motz *et al.*, 1997). Experiment 3 (Appendix 1, Table A) utilized a highly-modified apparatus developed to handle smaller samples. It consists of 6 cm lengths of 3 mm outside-diameter, 2 mm inside-diameter nickel gas-chromatography tubing sealed in 8 cm lengths of 9 mm outside diameter Vycor tubing. Samples were sealed inside the nickel tubes, in an argon atmosphere, using Tungsten Inert Gas (TIG) welding. These pyrolysis bombs were then sealed in the evacuated Vycor tubes, and heated as described above. Following pyrolysis the Vycor tubes were handled as breakseals to introduce contained hydrogen into the mass spectrometer, after which the bombs were punctured into a vacuum line for cryogenic CO_2 separation.

Differences between the three correction lines suggest that some of the processes which affect the isotopic composition of hydrogen produced are bomb-dependent. Correction lines in Figure 2.2 vary from $x = y$, which would be expected in the case of quantitative recovery of hydrogen. This may result from variations in diffusional efficiency related to bomb design or from memory effects in the Experiment 1 and 2

bombs, which were re-used. Whatever the reason, it is clear that each style of apparatus must be carefully calibrated before analyses of unknowns are undertaken.

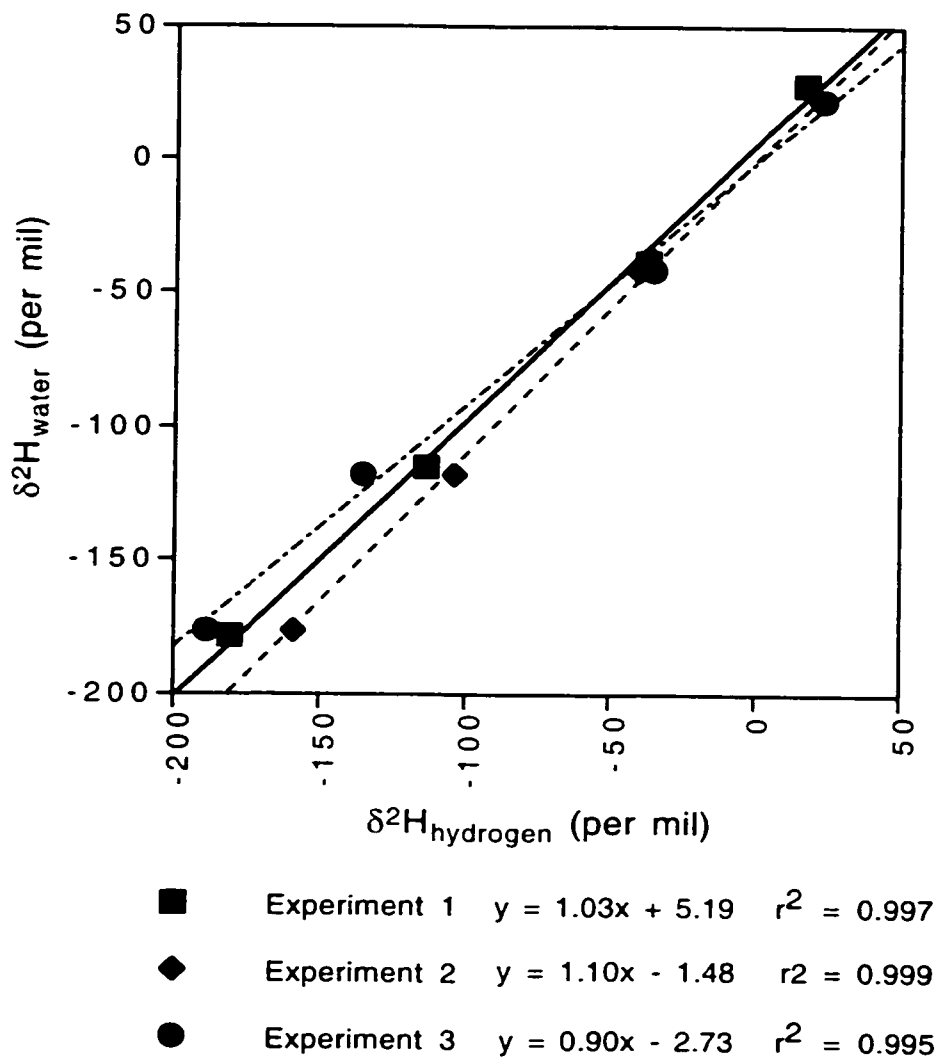


Figure 2.2: Relationship between $\delta^2\text{H}$ of pyrolysis hydrogen and known values for water standards using three different types of pyrolysis bombs. Error bars are smaller than symbols. Experiment 1 and 2 results were also reported by Motz *et al.*, (1997).

The $\delta^2\text{H}$ measurement precisions for water (n=4) and CH-7 (n=3) samples using the nickel-pyrolysis technique are ± 2.0 and $\pm 1.7\%$.

respectively which closely compare with the $\pm 2\%$ obtainable routinely with zinc reduction. The $\delta^2\text{H}$ results for wood cellulose ($-15.0 \pm 2.0\%$, $n=3$), chitin ($-7.6 \pm 1.1\%$, $n=4$), peptide ($-65.1 \pm 0.5\%$, $n=2$) and bound water in biotite mica ($-102.5 \pm 1.5\%$, $n=2$) show similar precisions, suggesting that this technique is applicable to a variety of materials.

The Experiment 1 and 2 apparatus were used for developing the equilibration method for chitin and cellulose and for some routine cellulose $\delta^2\text{H}$ and $\delta^{18}\text{O}$ analyses. The Experiment 3 apparatus was used for most chitin samples and all fossil cellulose samples.

The $\delta^{18}\text{O}$ -correction equations for the three apparatus were:

$$\text{Experiment 1: } \delta^{18}\text{O}_{\text{SMOW}} = 1.26\delta^{18}\text{O}_{\text{PDB}} + 51.50$$

$$\text{Experiment 2: } \delta^{18}\text{O}_{\text{SMOW}} = 1.26\delta^{18}\text{O}_{\text{PDB}} + 50.28$$

$$\text{Experiment 3: } \delta^{18}\text{O}_{\text{SMOW}} = 1.02\delta^{18}\text{O}_{\text{PDB}} + 43.11$$

A $\delta^{18}\text{O}_{\text{PDB}}$ to $\delta^{18}\text{O}_{\text{SMOW}}$ conversion was necessary because mass spectrometer working values are expressed relative to PDB. The Experiment 1 and 2 corrections are standard University of Waterloo Environmental Isotope Laboratory equations while the Experiment 3 correction was developed for this study, using a series of experiments with cellulose standards as follows:

True $\delta^{18}\text{O}_{\text{SMOW}}$ (‰)	Measured $\delta^{18}\text{O}_{\text{PDB}}$ (‰)
32 ± 0.14	-10.38 ± 0.01
27 ± 0.2	-17.01 ± 0.45
18.6 ± 0.63	-23.58 ± 0.28

Table 2.2: True and measured $\delta^{18}\text{O}$ values used in Experiment 3 apparatus correction. Each measured $\delta^{18}\text{O}_{\text{PDB}}$ value is an average of two analyses. Experimental values are listed in Appendix 1, Table A.

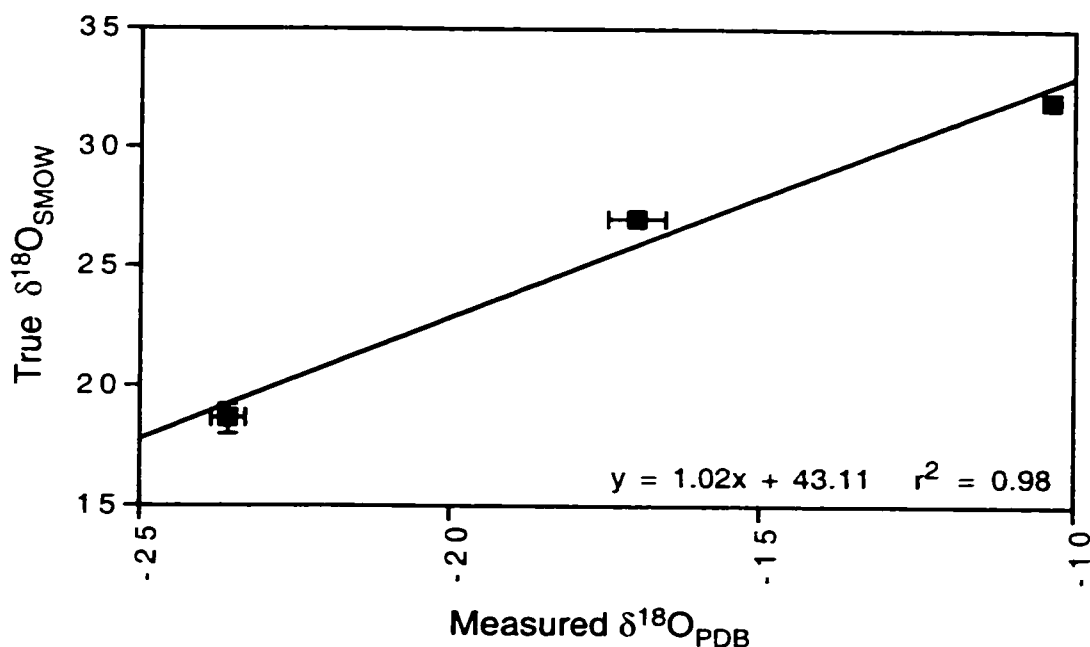


Figure 2.3: Derivation of $\delta^{18}\text{O}$ correction equation for Experiment 3 apparatus through linear regression of Table 2.2 data.

Precisions achieved for $\delta^{18}\text{O}$ measurements using the pyrolysis technique are ± 0.01 to $\pm 0.45\text{‰}$ ($n=2$) for modern cellulose, $\pm 0.82\text{‰}$ ($n=4$) for fossil cellulose and $\pm 0.62\text{‰}$ ($n=2$) for modern chitin using the Experiment 3 apparatus.

2.4: Sample-size correction:

Later work with very-small fossil samples showed that there is size-related fractionation inherent in pyrolysis, leading to depletions for minute samples. In the case of $\delta^2\text{H}$, this fractionation is possibly diffusion-related, as discussed previously, resulting from the preferential diffusion of lighter molecules through the nickel. For $\delta^{18}\text{O}$ the depletion may originate in the separation line where, in the course of transferring minute samples from bombs to breakseals, the lighter molecules move readily, while some heavier ones remain in the line.

In order to compensate for this, a series of experiments was run with progressively smaller amounts of a known homogenized chitin sample: the beetle *Tripodendron lineatum* from Kelsey Bay BC, $\delta^2\text{H}$ (with exchangeable H removed by nitration) = $-51.0 \pm 2.2\text{‰}$ (n=2), $\delta^{18}\text{O} = 16.67 \pm 0.67\text{‰}$ (n=4) (Table 2.3). Wet sample weights were used because the equilibration method of compensating for exchangeable hydrogen (Chapter 3) requires the use of wet samples and does not permit the determination of dry weights. Dry sample weight is approximately $10 \pm 2\%$ (n=9) of wet weight. Plotting the difference between the measured and known $\delta^2\text{H}$ values against sample size (Figures 2.4 and 2.5) resulted in the calibration equations:

$$\delta^2\text{H}_{\text{corrected}} = \delta^2\text{H}_{\text{measured}} - 1.74(\text{wet sample weight in mg}) + 39.40.$$

$$\delta^{18}\text{O}_{\text{corrected}} = \delta^{18}\text{O}_{\text{measured}} - 0.23(\text{wet sample weight in mg}) + 5.04$$

These corrections were used for all fossil chitin samples because of their small size. It is clear that for the smallest samples the $\delta^2\text{H}$ values are somewhat erratic, since the figure for a 4.6 mg sample is more depleted than that for a 1.3 mg sample. Nevertheless, this correction does provide a useful indication of size-related depletion, even though it must be accepted that the degree of error becomes greater as sample size is reduced.

Wet sample weight (mg)	Measured chitin $\delta^2\text{H}$ (‰)	Measured chitin $\delta^{18}\text{O}$ (‰)
24.8	-51.63	17.05
19.7	-50.13	16.17
7.1	-74.64	13.00
4.6	-90.84	12.81
1.3	-84.70	11.25

Table 2.3: Samples used for developing size correction. Dry sample weight is approximately $10 \pm 2\%$ (n=9) of wet weight.

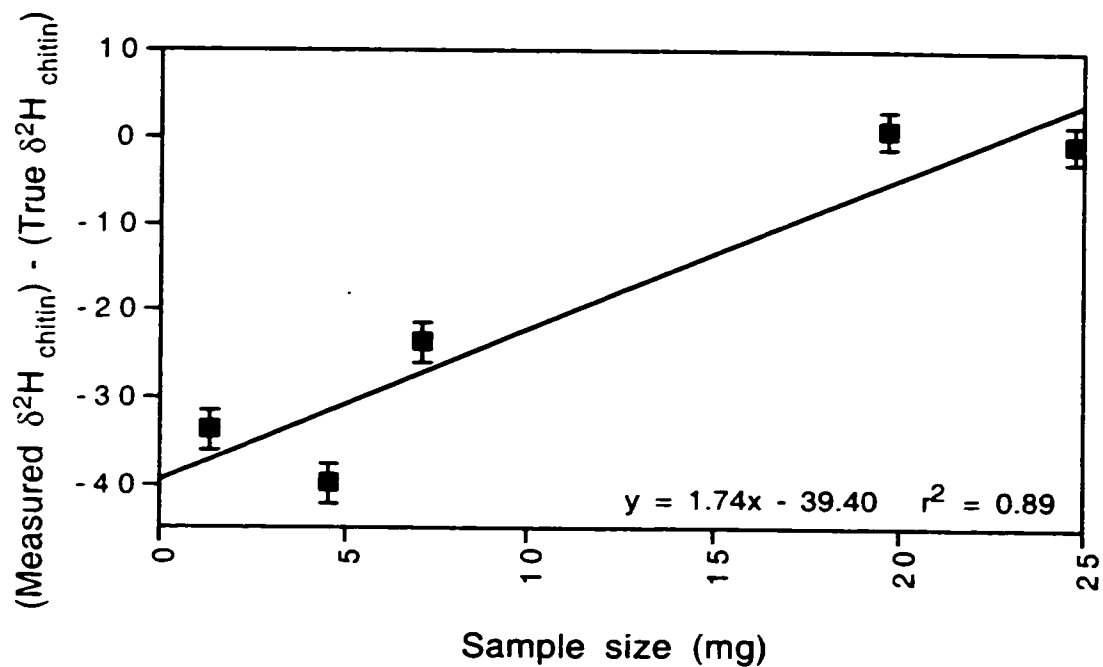


Figure 2.4: Plot used to derive correction of δ^{2H} for sample weight. This was necessary because measured δ^{2H} values became more depleted as sample size decreased. All samples are the same chitin with a known δ^{2H} of $-51.0 \pm 2.2\%$. Sample size is wet weight.

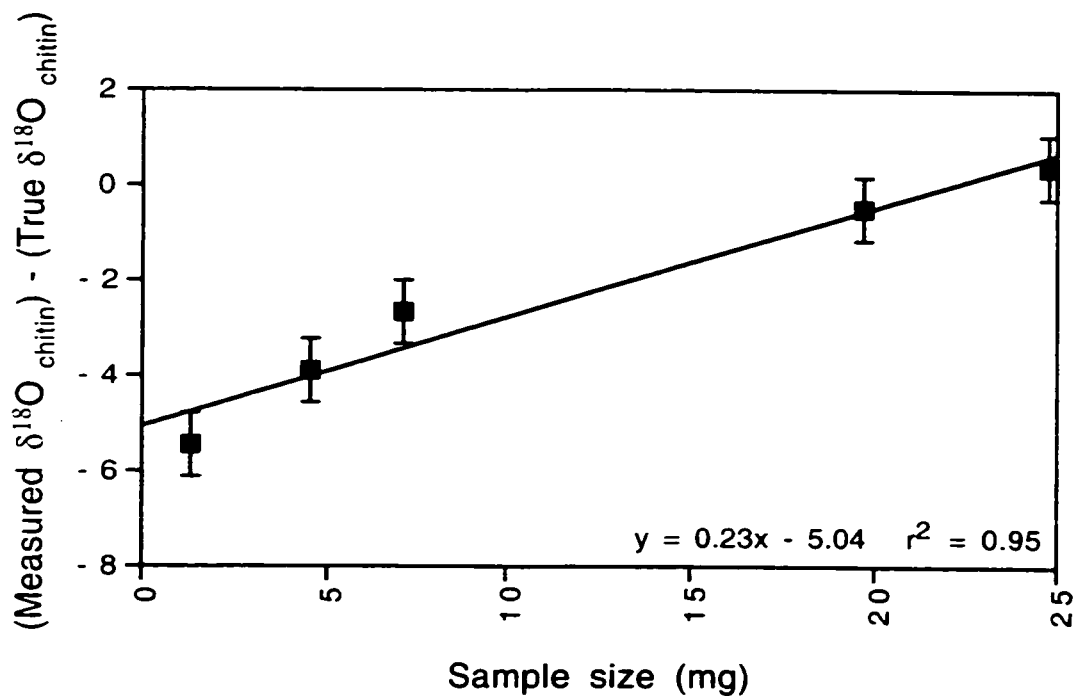


Figure 2.5: Plot used to derive correction of $\delta^{18}\text{O}$ for sample weight. This was necessary because measured $\delta^{18}\text{O}$ values became more depleted as sample size decreased. All samples are the same chitin with a known $\delta^{18}\text{O}$ of $-16.67 \pm 0.67\text{‰}$. Sample size is wet weight.

Chapter 3:

Compensating for exchangeable hydrogen:

One of the challenges in the use of $\delta^2\text{H}$ of fossil chitin as a paleoenvironmental indicator is the elimination of the portion of hydrogen in chitin (23% by weight) which is bonded to oxygen or nitrogen, rather than carbon. This is exchangeable with environmental hydrogen because hydrogen atoms bonded to highly electronegative atoms, such as oxygen, are very mobile and move rapidly between molecules (Moncrief and Jones, 1977). Therefore, non-carbon-bonded hydrogen is not conservative of the isotopic ratio at the time of chitin formation. A similar complication exists in cellulose $\delta^2\text{H}$ analysis since 30% (by weight) of the hydrogen in cellulose is oxygen-bonded and, therefore, exchangeable.

Miller (1984) used procedures developed for cellulose to eliminate exchangeable hydrogen by converting chitin to chitin nitrate for analysis. Schimmelmann and DeNiro (1986 a, b) eliminated exchangeable hydrogen by polycondensation of chitose to form furanes, which were then analyzed. Both these methods have the disadvantage of requiring relatively large samples: 5 to 10 mg for nitration and about 100 mg for the polycondensation technique, as well as elaborate preparation.

An alternative approach (Schimmelmann, 1991; Schimmelmann *et al.*, 1993) involves equilibration of chitin with water at high temperature in order to replace the exchangeable hydrogen with hydrogen of a known isotopic composition. This method allows measurement of hydrogen ratios on as little as 1 mg of chitin. It does, however, require the construction of an elaborate apparatus. It is also necessary to calculate the fraction of hydrogen exchangeable as well as the fractionation factor for the exchange between water and chitin if the isotopic ratio of the nonexchangeable hydrogen is to be determined.

Feng *et al.* (1993) developed a low-temperature exchange procedure similar, in principal, to Schimmelmann's (1991) method. It is more convenient than the earlier procedure because it does not require special apparatus or careful regulation of high temperatures during the exchange process. Calculations of the exchangeability and fractionation factor are required to allow determination of the isotopic ratio of nonexchangeable

hydrogen. Although this method was only tried on cellulose by the authors, it should also be applicable to chitin because of the chemical and structural similarities between the two compounds.

In analogous work with deer-bone collagen, Cormie *et al.* (1994 a, b) found sample material would equilibrate isotopically with atmospheric water vapour in the laboratory within 48 hours and no other special preparation was necessary. Once again, correction for the contribution of the equilibrated exchangeable hydrogen was necessary in order to arrive at δD for the non-exchangeable hydrogen.

The low-temperature-equilibration method (Feng *et al.* 1993) was used in these experiments because it allows the use of smaller sample sizes than the chemical techniques of removing exchangeable hydrogen. It is also simpler than the high-temperature-equilibration technique and allows more control over the isotopic composition of the exchanging hydrogen than does equilibration with ambient vapour. Equilibration also preserves the original oxygen content of the material, allowing for both δ^2H and $\delta^{18}O$ analyses on the same sample.

Adaptation of low-temperature equilibration to the needs of this research was done in two stages:

- i) Modification of the procedure to be compatible with nickel pyrolysis.
- ii) Standardization with chitin.

3.1: Procedure:

Standard techniques were employed to purify insects to chitin (Miller, 1984) (Appendix 2). Feng *et al.* (1993) found that soaking cellulose in a 17% NaOH solution for 15 to 18 hours at 0°C was necessary to swell the structure sufficiently to allow efficient exchange. Muzzarelli (1977) had previously found that much higher concentrations of NaOH were necessary to swell chitin to the same extent as cellulose and for these experiments chitin was soaked in a 38% NaOH solution for 15 to 18 hours at 0°C.

Following the NaOH treatment samples were rinsed with de-ionized water until neutral, then washed four times in the water to be used for equilibration in order to remove the isotopic signature of the rinse

water (Feng *et al.* 1993), then stored in the equilibration water. In order to do an experiment, excess water was decanted from the storage vessel, leaving the sample a moist pulp. When using the larger pyrolysis bombs (from Experiments 1 and 2 in the pyrolysis chapter) 100 to 150 mg portions of wet sample were loaded into sample boats made of 15 mm lengths of 6 mm outside-diameter quartz tubing, which were then inserted into the bombs and cracked with a steel rod. There is potential for exchange between oxygen in the quartz tube and that in the samples or reaction products. However, Hardcastle and Friedman (1974) ran a series of experiments examining oxygen isotopes in organic samples, reduced by diamonds in quartz tubes at 1250°C, and found no evidence of such exchange.

A 1.5 cc aliquot of equilibration water was then injected onto the samples. When using the small bombs (Experiment 3) 1 to 2 mg portions of wet sample were inserted directly into the bombs, which were then filled with equilibration water. Tests indicate that the dry weight of these samples is about 10% of their wet weight.

Loaded pyrolysis bombs were placed in a cooler containing an ice/water mixture, in order to maintain 0°C, to equilibrate for 48 hours. The bombs were then removed from the ice and immediately frozen in liquid nitrogen. Fast freezing is important because any change in temperature affects the equilibrium fractionation factor between the sample and water. Using this procedure, 0°C is maintained because there is no change in temperature during freezing, and once the sample is thoroughly frozen hydrogen exchange should essentially cease.

The frozen bombs were then placed in a vacuum dessicator attached to a freeze dryer for 48 to 72 hours to remove all water. The dessicator was then flooded with argon, the bombs sealed and the pyrolysis and gas separation procedures conducted as described in other chapters.

3.2: Standardization with chitin:

The equilibration procedure was developed for chitin using purified (Miller, 1984) chitin from the ambrosia beetle *Tripodendron lineatum* collected from Kelsey Bay British Columbia. This material has a $\delta^2\text{H}$ of $-51.0 \pm 2.2\text{‰}$, established by removing oxygen-bonded hydrogen using

nitration, followed by $\delta^2\text{H}$ analysis using nickel pyrolysis. Equilibration waters had $\delta^2\text{H}$ values of +21.4, -42.1, -73.5, -117.9 and -176.5‰, as determined by zinc reduction. Figure 3.1 is a plot of the data from these equilibration experiments (Appendix 1, Table B). It is clear, from Figure 3.1, that differing equilibration waters do have an effect on the chitin: with samples equilibrated with more depleted waters exhibiting lower $\delta^2\text{H}$ values. As well, the strong linearity and slope of less than one confirm that an isotope exchange reaction is occurring.

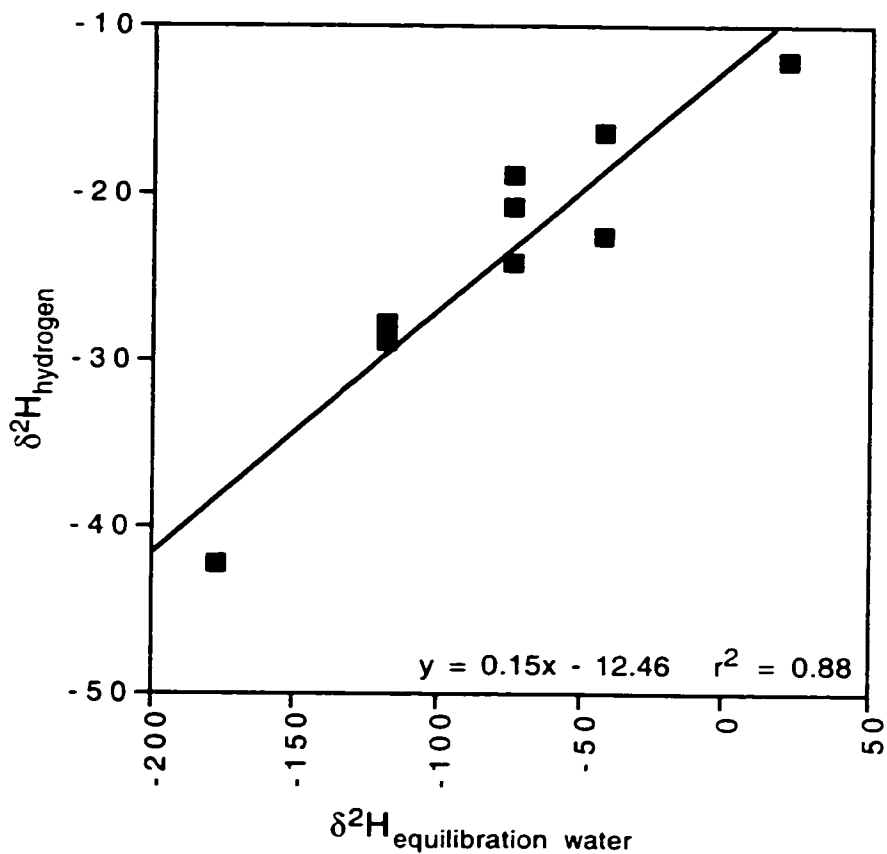


Figure 3.1: Relationship between $\delta^2\text{H}$ of pyrolysis hydrogen from equilibrated chitin and $\delta^2\text{H}$ of equilibration waters, verifying that hydrogen-isotope exchange has occurred.

Feng *et al.* (1993) described the relationship between total $\delta^2\text{H}$ of an equilibrated sample, the $\delta^2\text{H}$ of unexchangeable hydrogen and the exchangeability with the equation:

$$\delta^2\text{H}_T = (1 - x_E)\delta^2\text{H}_U + x_E \delta^2\text{H}_E \quad (3.1)$$

where $\delta^2\text{H}_T$ is the measured $\delta^2\text{H}$ of an equilibrated sample, x_E is the fraction of hydrogen that is exchangeable, $\delta^2\text{H}_U$ is the $\delta^2\text{H}$ of unexchangeable hydrogen and $\delta^2\text{H}_E$ is the $\delta^2\text{H}$ of exchanged hydrogen, which is related to the $\delta^2\text{H}$ of equilibration water by way of the equilibrium fractionation factor (α_{E-W}) through the equation:

$$\alpha_{E-W} = (\delta^2\text{H}_E + 1000) / (\delta^2\text{H}_W + 1000) \quad (3.2)$$

These two equations can be combined to produce the relationship:

$$\delta^2\text{H}_T = x_E\alpha_{E-W}\delta^2\text{H}_W + (1 - x_E)\delta^2\text{H}_U + 1000x_E(\alpha_{E-W} - 1) \quad (3.3)$$

which can be rearranged into the expression:

$$\delta^2\text{H}_U = [\delta^2\text{H}_T - x_E\alpha_{E-W}\delta^2\text{H}_W - 1000x_E(\alpha_{E-W} - 1)] / (1 - x_E) \quad (3.4)$$

which will give the $\delta^2\text{H}_U$ from a measured $\delta^2\text{H}_T$ provided $\delta^2\text{H}_W$, x_E and α_{E-W} are known. In order to determine these factors for cellulose, Feng *et al.* (1993) assumed that $\delta^2\text{H}_U$ of an equilibrated sample is equal to $\delta^2\text{H}_{C-H}$ ($\delta^2\text{H}$ of carbon-bonded hydrogen) determined by nitration and rearranged equation 3.3 to produce:

$$\begin{aligned} (\delta^2\text{H}_T + 1000) / (\delta^2\text{H}_{C-H} + 1000) = \\ x_E\alpha_{E-W}(\delta^2\text{H}_W + 1000) / (\delta^2\text{H}_{C-H} + 1000) + (1 - x_E) \end{aligned} \quad (3.5)$$

Figures for x_E and α_{E-W} can be derived from the slope and intercept of this equation after a series of equilibration experiments to determine $\delta^2\text{H}_T$ for a variety of $\delta^2\text{H}_W$ values. Feng *et al.* (1993) used this technique to arrive at an average x_E of 25.5% and α_{E-W} of 1.233 for cellulose. Exchange is probably incomplete because the structure of the cellulose molecule

renders some oxygen-bonded hydrogen inaccessible. Since the hydrogen is not completely exchanged, $\delta^2\text{H}_\text{U}$ and $\delta^2\text{H}_\text{C-H}$ are not equal, resulting in sample-dependent variations in $\alpha_{\text{E-W}}$ because of differences in x_{E} . They calculated that using average values for x_{E} and $\alpha_{\text{E-W}}$ would lead to a standard error of $\pm 4.4\text{‰}$ and compensated for this by using nitration and equilibration experiments to calibrate the technique for each wood specimen. This is feasible when performing tree-ring studies in which there are many samples from one tree, meaning each calibration is valid for several samples. However, it is clearly impractical for fossil studies, particularly those with chitin where only a few milligrams of material from a mixed-species assemblage may be available. Because of this it was decided to use average values for exchangeability and fractionation for cellulose from Feng *et al.* (1993) and determinations of chitin x_{E} and $\alpha_{\text{E-W}}$ from the *T. lineatum* experiments for this study.

To calculate these factors, the $\delta^2\text{H}_\text{T}$ and $\delta^2\text{H}_\text{W}$ values for *T. lineatum* from Figure 3.1, along with the $\delta^2\text{H}_\text{C-H}$ of -51.0‰ were used in equation 3.5 and plotted in Figure 3.2. This produces an x_{E} of 11.3% from the intercept $(1 - x_{\text{E}})$ and a $\alpha_{\text{E-W}}$ of 1.292 from the slope $(x_{\text{E}}\alpha_{\text{E-W}})$.

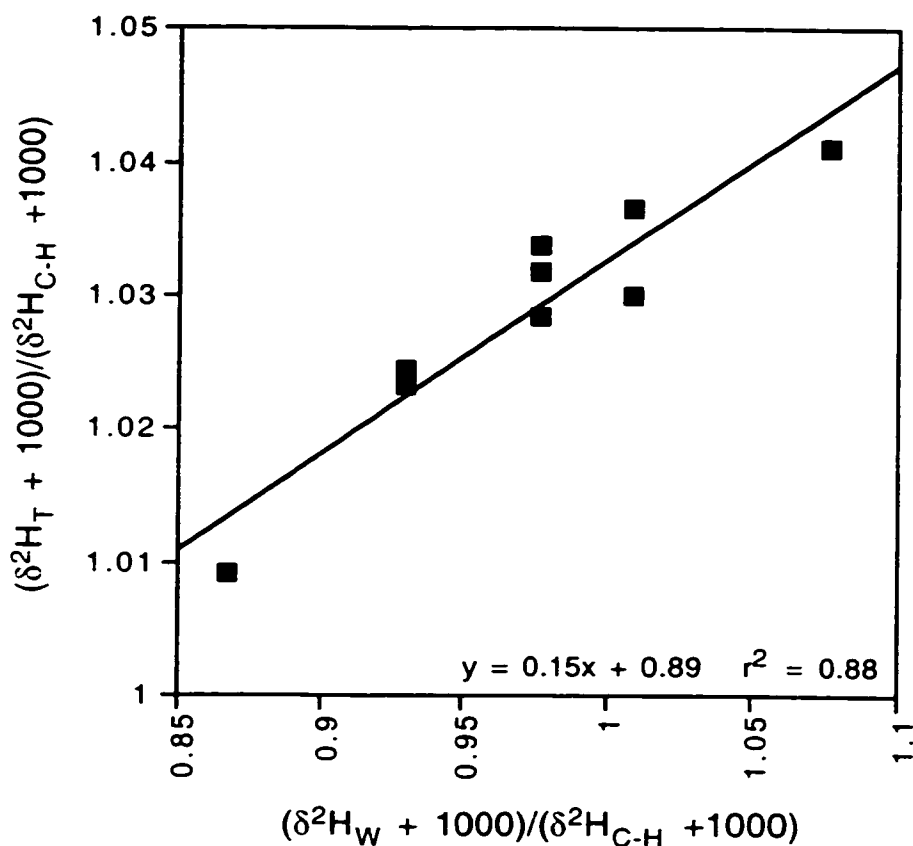


Figure 3.2: Relationship between the apparent fractionations between δ^2H_T and δ^2H_{C-H} , and δ^2H_W and δ^2H_{C-H} . This allows calculation of the exchangeability (x_E) from the intercept ($1 - x_E$) and the equilibrium fractionation factor α_{E-W} from the slope ($x_E\alpha_{E-W}$) in equation 3.5.

This exchangeability is much lower than the theoretical 23% as well as the 15.8 to 17.2% reported by Schimmelmann (1991) and Schimmelmann *et al.* (1993). A lower apparent exchangeability in the present study could be partially due to the lower temperatures employed (Schimmelmann, 1991). Schimmelmann (1991) derived x_E from a linear regression of the expression:

$$\delta^2H_T = \delta^2H_U(1 - x_E) + x_E(\delta^2H_W + \epsilon) \quad (3.6)$$

which expresses δ^2H_T as the sum of the δ^2H values of the exchanged and unexchanged hydrogen pools (where ϵ is simply the arithmetic isotopic separation between exchangeable hydrogen and water in parts per thousand), rearranged to produce the linear equation:

$$\delta^2H_T = x_E \delta^2H_W + (\delta^2H_U - x_E \delta^2H_U + x_E \epsilon) \quad (3.7)$$

When δ^2H_T is plotted against δ^2H_W the slope of the resulting regression is x_E when equation 3.7 is applied. However, it is clear from equation 3.3 that the slope of δ^2H_T versus δ^2H_W is the product of x_E and α_{E-W} . Therefore, if the slopes of 0.158 and 0.172 are viewed as $x_E \alpha_{E-W}$, rather than x_E , they can be divided by the α_{E-W} of 1.292 derived above to yield a x_E range of 12.2 to 13.3%. Clearly, the exchangeability achieved by Schimmelmann (1991) is similar to that in the current procedure, despite the higher temperatures used in the earlier work. Furthermore, the use of equation 3.7 to interpret a linear relationship is not strictly valid, since ϵ , as defined by $\epsilon = \delta^2H_E - \delta^2H_W$, is not constant, as demonstrated by the following:

$$\alpha_{E-W} = (\delta^2H_E + 1000) / (\delta^2H_W + 1000)$$

which can be arranged to produce

$$\delta^2H_E = \alpha_{E-W} \delta^2H_W + (\alpha_{E-W} - 1)1000$$

so that

$$\delta^2H_E - \delta^2H_W = (\alpha_{E-W} - 1) \delta^2H_W + (\alpha_{E-W} - 1)1000$$

therefore

$$\epsilon = (\alpha_{E-W} - 1) \delta^2H_W + (\alpha_{E-W} - 1)1000$$

and varies with δ^2H_W .

For routine equilibrations a supply of laboratory filtered de-ionized water was established. The $\delta^2\text{H}$ of this water is $-68.9 \pm 0.8\text{‰}$ ($n=3$) which provides the $\delta^2\text{H}_w$ value. Using the constants determined in the foregoing discussion, x_E of 25.5% and α_{E-w} of 1.233 for cellulose and x_E of 11.3% and α_{E-w} of 1.292 for chitin, and the -68.90‰ $\delta^2\text{H}_w$ value in equation (3.4) produces the following $\delta^2\text{H}_U$ expressions:

$$\delta^2\text{H}_{U(\text{cellulose})} = (\delta^2\text{H}_T - 37.75)/0.745 \quad (3.8)$$

$$\delta^2\text{H}_{U(\text{chitin})} = (\delta^2\text{H}_T - 22.94)/0.887 \quad (3.9)$$

Results are $\pm 3.4\text{‰}$ ($n=9$) for chitin and $\pm 4.1\text{‰}$ ($n=8$) for cellulose. Note that these precisions include the approximately $\pm 2\text{‰}$ error inherent in the pyrolysis procedure, so the error attributable to equilibration is smaller than these figures suggest.

Chapter 4:

The relationship between oxygen and hydrogen isotopes in chitin and environmental parameters:

The oxygen and hydrogen isotopic signatures of insect chitin are believed to be derived from that of the insects' food, modified by metabolic processes (Miller, *et al.*, 1988, Schimmelmann and DeNiro, 1986a). As outlined in the introduction, the results of past work have been contradictory: Miller (1984) and Miller, *et al.* (1988) show good correlations between $\delta^2\text{H}$ of chitin and the corresponding isotopic ratio in environmental water and temperature; however, Schimmelmann and DeNiro (1986a) found a poor correlation between modern arthropod chitin $\delta^{18}\text{O}$ and $\delta^2\text{H}$ and the isotopic content of environmental water. Since the feeding habits of most insect species are not well known, separating isotopic effects related to dietary preferences from those related to climate and hydrology has been difficult. My goal is to elucidate how the interrelationships among environmental water, food source and climatic factors affect the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ signatures of insect chitin, using a suite of modern samples, in order to calibrate transfer functions that can be employed to infer paleo-environmental information from fossil insect material.

4.1: Method:

In order to clarify the isotopic links between the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of insect chitin, the insects' food, climate and environmental water, insect and wood samples were collected from eleven localities along a transect in Canada from northern Ontario to the southern Northwest Territories (sites 1, 2 and 4 through 12 in Figure 4.1 and Tables 4.1 to 4.4) during the summer of 1993. The insects chosen for study (with the exception of site two) were bark beetles (Coleoptera, Scolytidae) (Arnett, 1968) which live in and feed on the inner bark of trees. They were selected because the insects and their food could be collected simultaneously and, since they are herbivores, there should be no isotopic effects related to multiple trophic levels. Furthermore, bark beetles are common in fossil-insect sites, where they generally occur with wood, allowing for extension of the analysis of modern materials into the fossil record.

Insects analyzed comprised four species of Scolytidae from three genera. Site 2 insects were Cerambycidae (another family of wood-consuming beetles) (Arnett, 1968), collected because no Scolytidae could be found.

In order to provide data from a wider range of environments, Coleoptera from three other localities (sites 3, 13 and 14) were also analyzed for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ content. These taxa were not bark beetles and samples of their host material were not available for analysis.

Wood samples consisted of the inner bark from which the beetles were collected, except for site 2 where heartwood was used. All wood samples were *Picea* spp., with the exception of sites 2 and 5 which were *Pinus* spp. Published techniques were employed to purify insects and wood to chitin (Miller, 1984) and cellulose (Green, 1963) respectively. Nickel pyrolysis (Edwards, *et al.*, 1994) was used to generate CO_2 for $\delta^{18}\text{O}$ measurements. The $\delta^2\text{H}$ values were determined for chitin and cellulose using nickel pyrolysis (Mutz, *et al.*, 1997) combined with equilibration to compensate for exchangeable hydrogen. The isotopic composition of local environmental water (precipitation) was estimated from published information (Rozanski, *et al.*, 1993). Canadian temperature and growing-season relative humidity (RH) were taken from Environment Canada (temperature, 1982a and 1993a-d; humidity, 1984 and 1993a-d) publications covering the 1951-1980 and 1961-1990 periods. United States figures were from Baldwin (1968) and Ruffner and Bair (1981) and are an average of values for 1931-1959 and 1941-1970 respectively. Growing-season relative humidity was calculated as per Edwards and Fritz (1986): Humidities were averaged for the 0400 to 1900 hour period (local time) for the growing season (indicated in the captions). Isotopic and environmental data for all sites can be found in Tables 4.1 to 4.5.

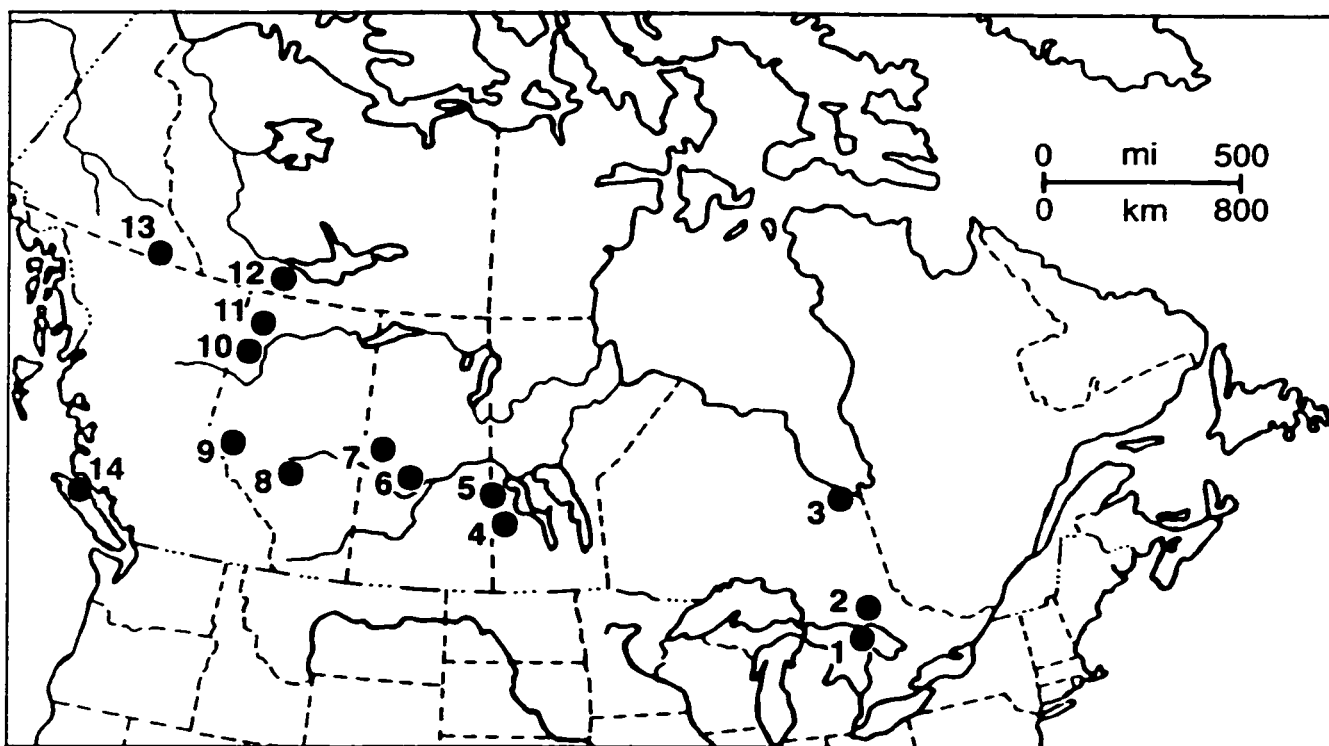


Figure 4.1: Insect sampling sites in southern Canada.

In order to set the stage for the determination of isotopic effects, it is instructive to look at how $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of chitin and cellulose, for the 14 sites in Figure 4.1, are situated with respect to the global meteoric water line (Figure 4.2) (Craig, 1961). This illustrates how the isotopic content of insect and plant material is offset from environmental water by biological fractionation and the effects of evaporative enrichment (Edwards, 1993 and references in the introduction of this work). The discussions in this chapter will attempt to quantify the fractionation factors causing this offset in chitin and use this knowledge to model the mechanisms involved.

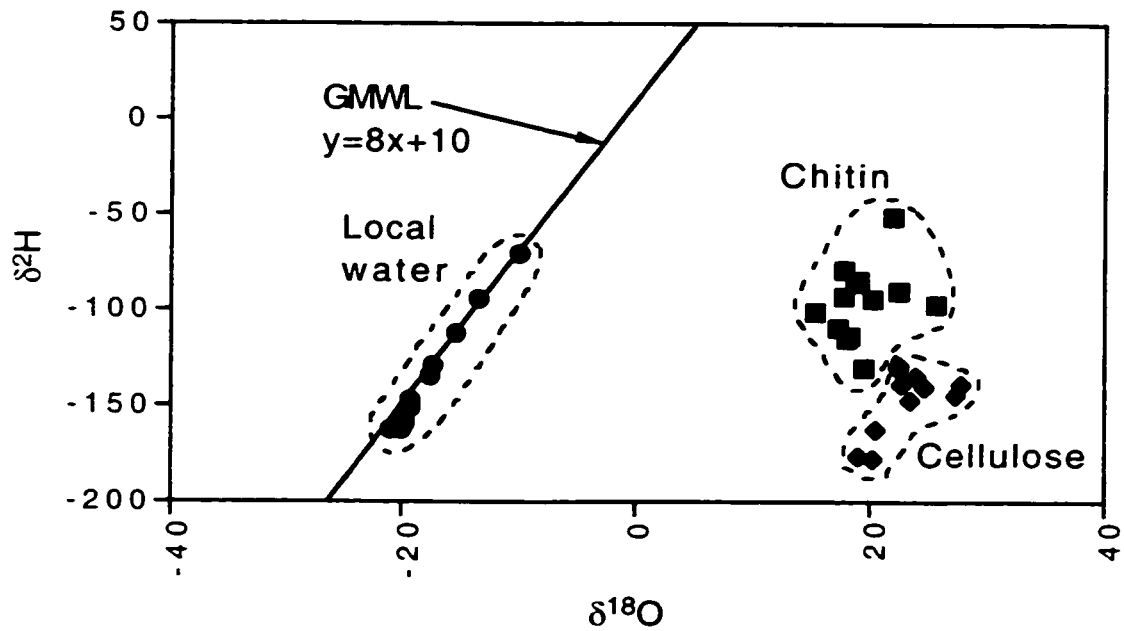


Figure 4.2: Relationship of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ for chitin and cellulose to the global meteoric water line (GMWL) (Craig, 1961). Note that the isotopic compositions of both biological materials deviate strongly from that of local meteoric water because of fractionations occurring during evapotranspiration from plants and biological processes in plants and insects.

4.2: Oxygen discussion:

Site Number	Site Name	Taxon	Diet
1	West Bay, Ont.	<i>Ips pini</i>	wood
2	Espanola, Ont.	<i>Monochamus scutellatus</i>	wood
3	Moosonee, Ont.	<i>Phaedon</i> sp.	plants
4	Pine River, Man.	<i>Dendroctonus rufipennis</i>	wood
5	Swan River, Man.	<i>Ips pini</i>	wood
6	Prince Albert, Sask.	<i>Ips pini</i>	wood
7	Glaslyn, Sask.	<i>Ips pini</i>	wood
8	Elk Isl. Nat. Park, Alta.	<i>Ips pini</i>	wood
9	Grand Prairie, Alta.	<i>Ips pini</i>	wood
10	64 km. N. of Manning, Alta.	<i>Polygraphus rufipennis</i>	wood
11	High Level, Alta.	<i>Ips latidens</i>	wood
12	Hay River, N.W.T.	<i>Polygraphus rufipennis</i>	wood
13a	Watson Lake, Y.T.	<i>Arhopalus foveicollis</i>	wood
13b	Watson Lake, Y.T.	<i>Monochamus scutellatus</i>	wood
14a	Kelsey Bay, B.C.	<i>Rhagium inquisitor</i>	wood
14b	Kelsey Bay, B.C.	<i>Nicrophorus defodiens</i>	carrion
14c	Kelsey Bay, B.C.	<i>Cucujus clavipes</i>	fungus
14d	Kelsey Bay, B.C.	<i>Tripodendron lineatum</i>	fungus

Table 4.1: Sites and taxa used for $\delta^{18}\text{O}$ analyses.

Site Number	Chitin $\delta^{18}\text{O}$ (‰ \pm 0.62)	Cellulose $\delta^{18}\text{O}$ (‰ \pm 0.45)	Water $\delta^{18}\text{O}$ (‰)	Annual Temperature (°C \pm 1)	Growing-Season Relative Humidity (%)
1	22.51	27.51	-13.2	4.7	72 \pm 4
2	25.62	27.87	-13.2	3.5	66 \pm 6
3	18.06		-15.2	-1.3	73 \pm 4 *
4	18.74	24.1	-17.2	1.7	64 \pm 5
5	20.35	22.48	-17.5	1.4	64 \pm 5
6	17.88	24.68	-19.3	0.5	64 \pm 6
7	18.97	23.45	-19.3	-0.2	60 \pm 5
8	18.95	22.42	-19.1	2.6	61 \pm 7
9	18.27	22.72	-19.3	1.6	61 \pm 4 *
10	17.72	20.5	-19.6	0.7	63 \pm 4 *
11	17.45	20.29	-19.8	-0.9	63 \pm 4 *
12	15.43	19.16	-2.0	-3.4	67 \pm 3 *
13a	17.83		-20.9	-3.1	57 \pm 5 *
13b	21.29		-20.9	-3.1	57 \pm 5 *
14a	24.92		-9.9	8.5	83 \pm 3
14b	23.2		-9.9	8.5	83 \pm 3
14c	23.68		-9.9	8.5	83 \pm 3
14d	16.67		-9.9	8.5	83 \pm 3

Table 4.2: RH values are averages for 0400 to 1900 local time for May through September and June through August (*). Water $\delta^{18}\text{O}$ precision is estimated to be on the order of $\pm 0.5\text{‰}$.

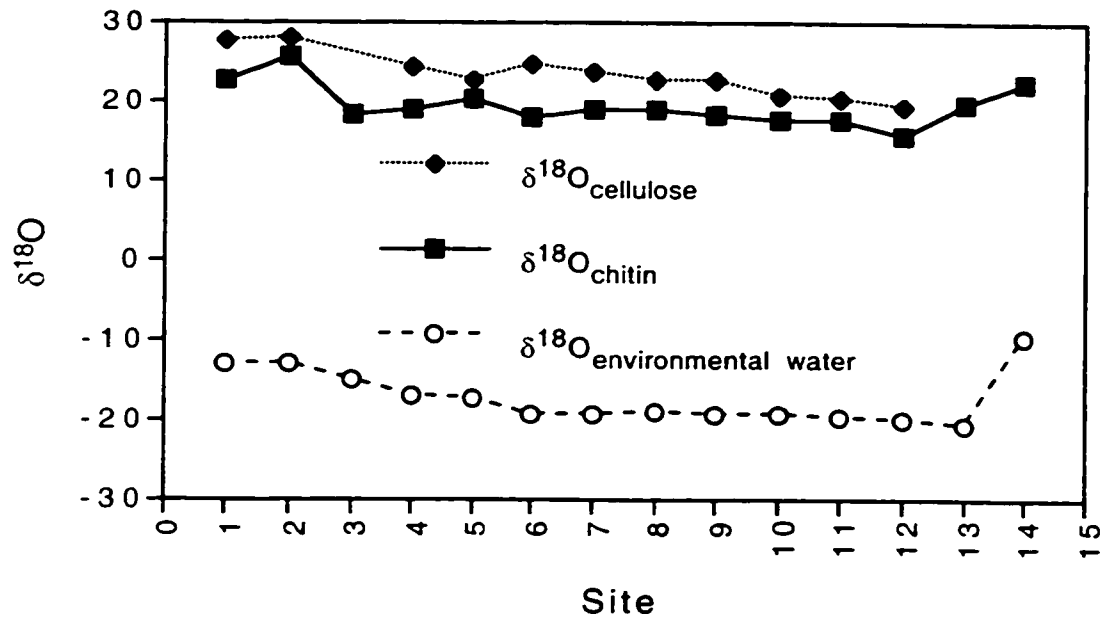


Figure 4.3: $\delta^{18}\text{O}$ values of chitin, wood and environmental water plotted against sites from Figure 4.1. $\delta^{18}\text{O}_{\text{chitin}}$ values are averaged for sites with multiple taxa. The roughly parallel trends suggest that environmental water $\delta^{18}\text{O}$ is influencing the isotopic compositions of chitin and cellulose.

The $\delta^{18}\text{O}$ values of these three materials showed significant parallelism (Figure 4.3) suggesting that the isotopic content of insect chitin does indeed reflect that of environmental water, probably as a result of the incorporation into chitin of material synthesized from water in the food trees. Oxygen isotopic values for cellulose and chitin exhibit enrichment over those for water, likely due to fractionation from vital effects and evapotranspirative enrichment related to RH (Edwards and Fritz, 1986). Chitin is enriched over environmental water an average of $36.79 \pm 2.20\text{‰}$ while cellulose is enriched by an average of $41.15 \pm 1.39\text{‰}$ over environmental water. Chitin from marine arthropods has been found to be enriched in ^{18}O by about 26‰ over sea water (Schimmelmann and DeNiro, 1986a), while oxygen from a plant's environmental water is enriched by approximately 27 to 28‰ during

cellulose synthesis (Epstein *et al.*, 1977; Edwards *et al.*, 1985; DeNiro and Cooper, 1989; Yakir and DeNiro, 1990; Yakir, 1992). Enrichments beyond these figures are likely due to evapotranspirative effects. It is well known that these effects in plants vary depending on RH (Edwards and Fritz, 1986; White *et al.*, 1994; Buhay, *et al.*, 1996; Lipp, *et al.*, 1996; Saurer *et al.*, 1997; Anderson *et al.*, 1998), with enrichment increasing in drier environments due to increased evapotranspiration through the leaves, which concentrates ^{18}O in the leaf water.

Sites with multiple taxa (13 and 14) demonstrate the $\delta^{18}\text{O}_{\text{chitin}}$ variability that can be present in an assemblage, likely due to different feeding habits. Intriguingly though, three species from site 14, *Rhagium inquisitor*, *Nicrophorus defodiens*, *Cucujus clavipes*, which have different food sources have similar $\delta^{18}\text{O}_{\text{chitin}}$ values. For the purpose of discussion, $\delta^{18}\text{O}_{\text{chitin}}$ values for multi-taxa sites are averaged on data plots. This approximates what would actually happen during analysis of a fossil site, where chitin samples are likely to be bulk material of partially unknown taxonomic content due to the low volume available.

The parallel trend between chitin and water $\delta^{18}\text{O}$ exhibited in Figure 4.3 translates into a moderate linear relationship (Figure 4.4). The relatively-poor correlation between $\delta^{18}\text{O}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{environmental water}}$ is not surprising: As can be seen in Table 4.2, RH varies widely among the sites, with driest conditions in the prairies (4-12) and at Watson (13) Lake, and humid environments at sites near water: West Bay (1), Moosonee (3) and Kelsey Bay (14). This wide range of RH would lead to significant variability in evaporative enrichment in the plant-produced carbohydrates, which are the precursors of chitin. Schimmelmann and DeNiro (1986a) found an even poorer correlation between $\delta^{18}\text{O}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{environmental water}}$ in semi- and non-aquatic arthropods, obtaining a correlation coefficient close to zero. However, their results did show a significant enrichment in $\delta^{18}\text{O}_{\text{chitin}}$ over water, with a fractionation factor ($^{18}\alpha_{\text{cellulose-water}}$) of approximately 1.0240.

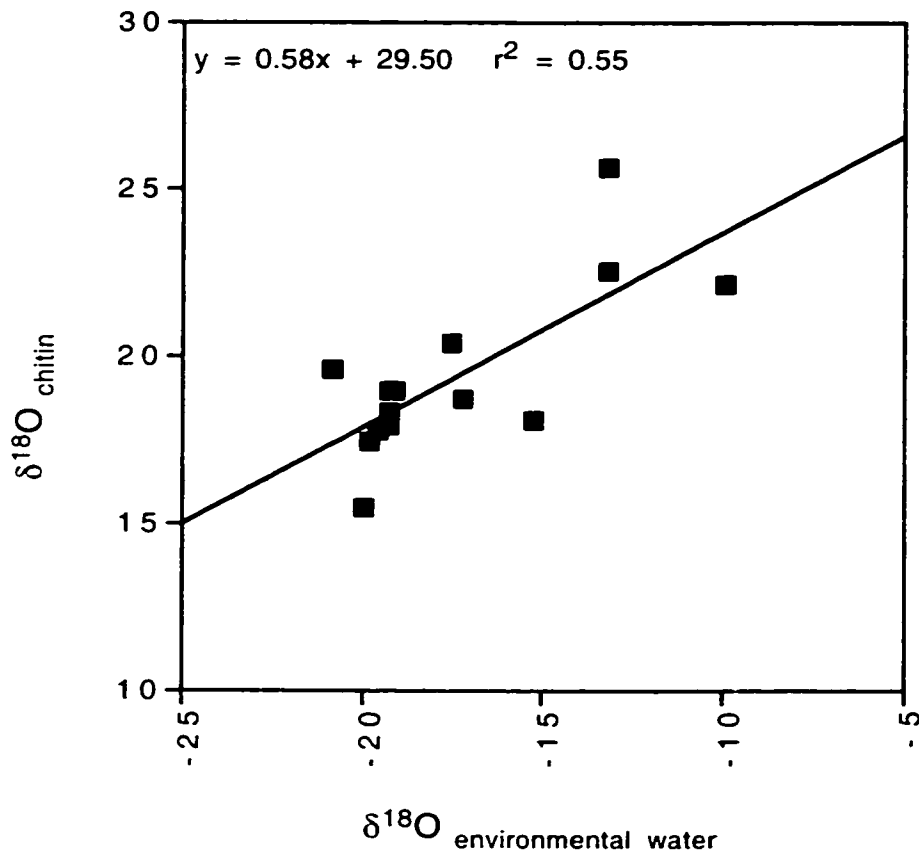


Figure 4.4: $\delta^{18}\text{O}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{environmental water}}$. This demonstrates that environmental water does influence the isotopic composition of chitin, although other factors are causing scatter in the relationship.

The effects of RH on $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ are illustrated in Figure 4.5, which shows the relationship between $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ ($\delta^{18}\text{O}_{\text{chitin}} - \delta^{18}\text{O}_{\text{environmental water}}$), $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ ($\delta^{18}\text{O}_{\text{cellulose}} - \delta^{18}\text{O}_{\text{environmental water}}$) and RH trends among the sites. Co-variant trends are formed between the three plots when the scale for $\Delta^{18}\text{O}$ is reversed, indicating an inverse relationship.

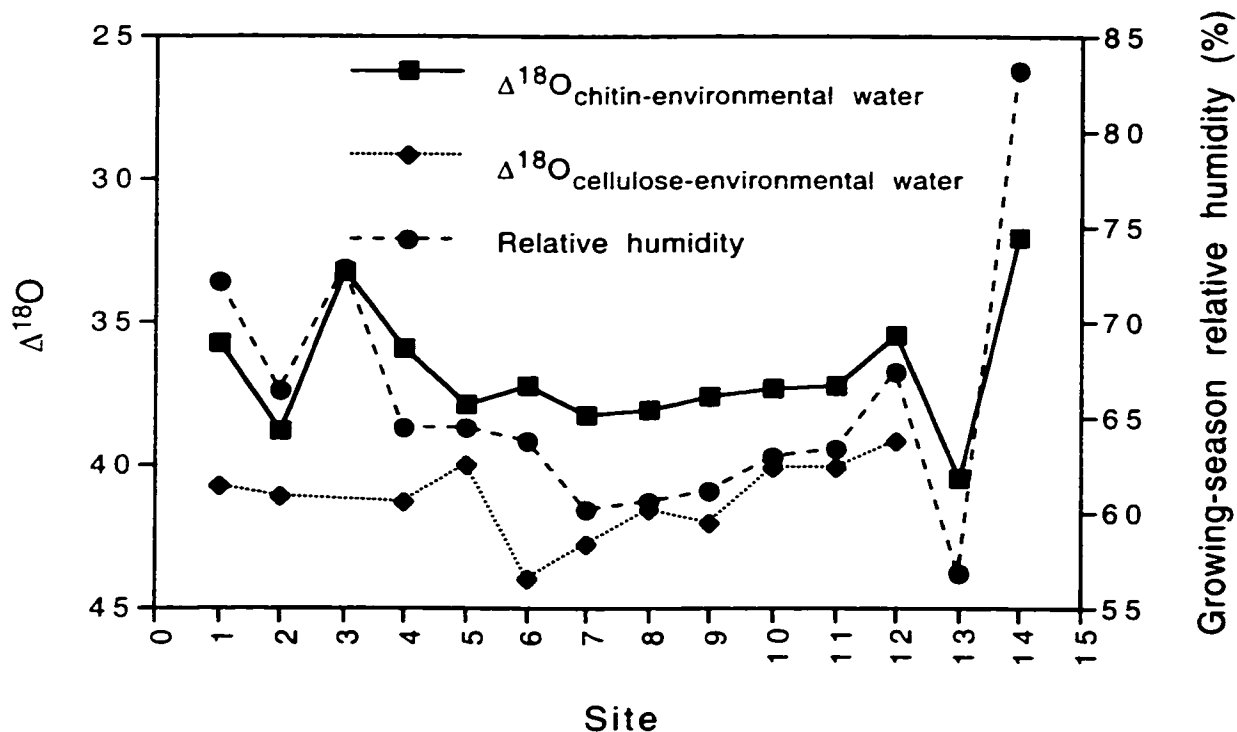


Figure 4.5: $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$, $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ (isotopic separations) and RH versus site. Note that $\Delta^{18}\text{O}$ scale is inverted to emphasize the inverse relationship between isotopic separation and relative humidity.

Analyses of chitin biosynthesis from glucose (Chippendale, 1978; Miller, 1984), which the insect would obtain from the food plant, suggest that all oxygen in chitin is derived from glucose molecules. Therefore, since evapotranspirative effects should be inherited from carbohydrates the insects consume, the difference between the values of $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ would result entirely from biochemical fractionation factors. The average $\delta^{18}\text{O}$ depletion of chitin with respect to cellulose in this study is $3.94 \pm 1.44\text{‰}$ with a range of 2.13 to 6.80‰. Schimmelmann and DeNiro (1986a) found that marine chitin was enriched over environmental water by $26.3 \pm 1.2\text{‰}$. Epstein *et al.*, (1977) reported cellulose of aquatic plants is enriched by $27.6 \pm 0.4\text{‰}$ over the water in

which they grew, while Burk and Stuiver (1981) found enrichments of 26 or 27‰ for terrestrial plants from six sites, normalized for 100% RH. Edwards, *et al.* (1985) calculated an average enrichment of 28.2 ± 0.3 ‰ for 11 sites (including the six from Burk and Stuiver, 1981), although the values are somewhat dependent on assumed temperature and leaf water isotopic composition. Yakir and DeNiro (1990) found oxygen isotopic fractionations of 26 or 27‰ during the synthesis of cellulose. These figures suggest that biochemical fractionation causes enrichments of similar magnitude in cellulose and chitin.

Plotting the $\Delta^{18}\text{O}$ values against RH (Figure 4.6) produces a strong linear trend for $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ and a weak one for $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$. Application of a line ($y = -0.29x + 60.13$) with the same slope as the $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ versus RH regression and a fixed enrichment of 3.94‰ (the average $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$) to the $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ versus RH points produces a reasonable fit. When sites 1 and 6 (circled in Figure 4.6) are removed from the $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ versus RH regression (Figure 4.7), the linear fit is improved. These points could be regarded as outliers because they lie significantly outside the cluster of the other nine points in Figure 4.6 and deviate from the trends in Figure 4.5. A linear regression through these two points produces the equation $y = -0.39x + 69.21$, which is roughly parallel to a line through the other nine points (Figure 4.7) but enriched by about 7.5‰. This suggests that the cellulose samples from sites 1 and 6 were subject to anomalous enrichment, possibly related to unusual growing conditions, making them poor candidates for inclusion in an isotope model for general application. Edwards (1993) described one such case of a tree from an Indian site (Ramesh *et al.*, 1985) in which samples from two different radii produced different inferred (from the Edwards and Fritz, 1986, model) $\delta^{18}\text{O}_{\text{environmental water}}$ and RH values, possibly because one side of the tree was exposed to evaporated water which did not fall on the meteoric water line, thus invalidating this assumption in the model.

Disregarding sites 1 and 6, the average $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ becomes 3.50 ± 1.10 ‰. The dashed line in Figure 4.7 is constructed using this $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ value, such that $y = -0.29x + 59.69$, which is close to the

actual linear regression. A 3.50‰ $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ value is used in all subsequent calculations.

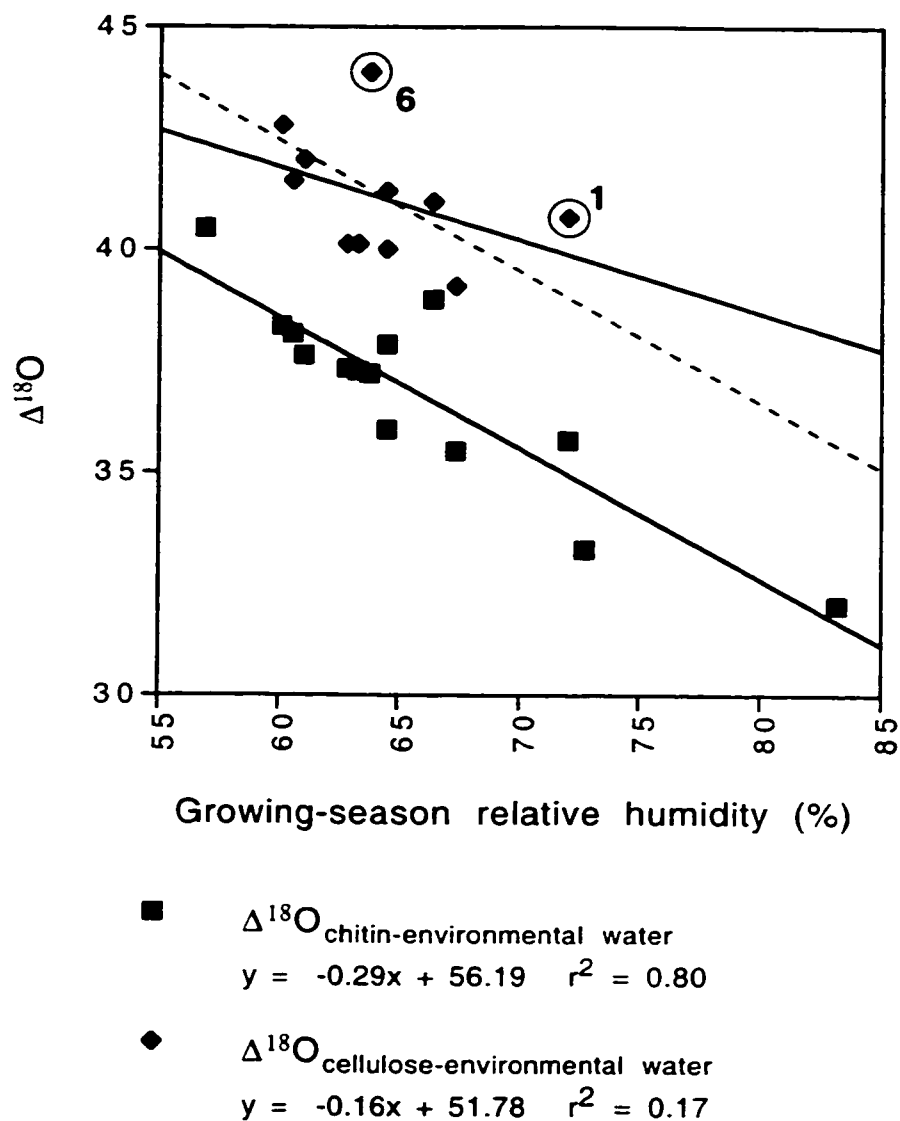
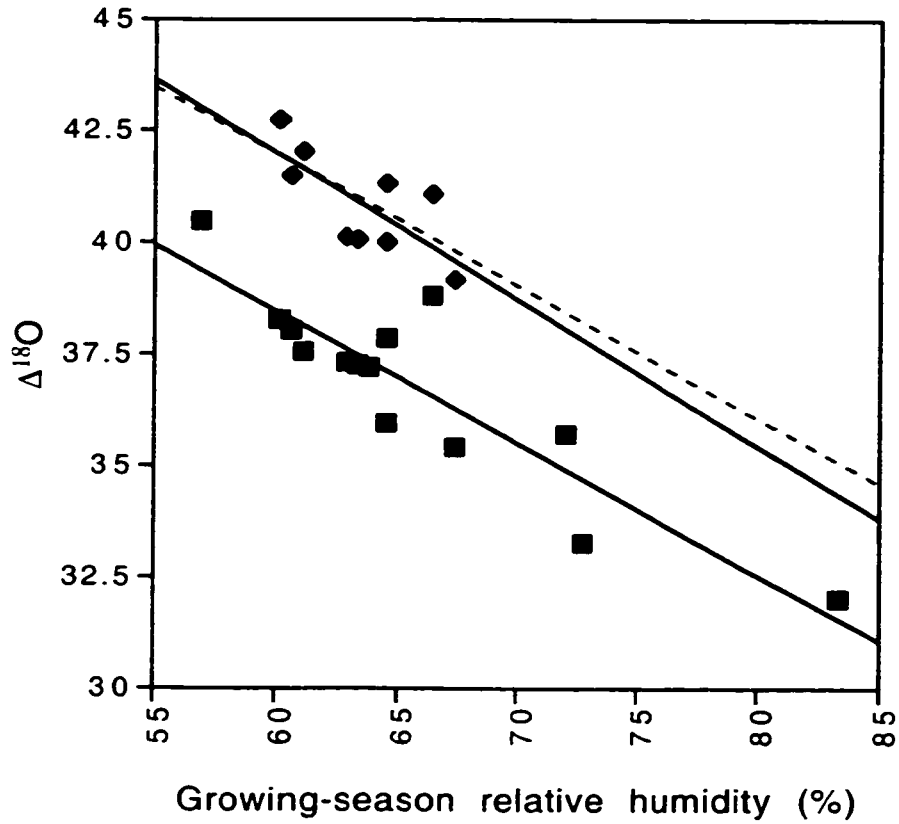


Figure 4.6: $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ and $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ versus RH. The dashed line, $y = -0.29x + 60.13$, is generated by assuming the same slope as the $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ versus RH plot and adding a fixed $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ enrichment of 3.94‰ (the average value for all sites) to the intercept. Points for sites 1 and 6 are circled.



- $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$
 $y = -0.29x + 56.19 \quad r^2 = 0.80$
- ◆ $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$
 $y = -0.33x + 61.69 \quad r^2 = 0.53$

Figure 4.7: $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ and $\Delta^{18}\text{O}_{\text{cellulose-environmental water}}$ versus RH with sites one and six removed. The dashed line is $y = -0.29x + 59.69$, based on a $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ enrichment of 3.50‰.

A slope of approximately -0.3 for the best-fit lines in Figure 4.7 is close to the -0.36 slope expected for Craig-type evaporative-enrichment response of leaf water (Craig and Gordon, 1965), if the equilibrium and kinetic fractionation factors derived below are used. This assumes the leaf is a water reservoir with constant input from stem water and output via evaporation through the stomata. Evaporation enriches the leaf water and this effect is passed on to sugars synthesized in the leaves. Burk and Stuiver (1981) show a 0.25‰/% of RH response for six sites in the United States, while Edwards and Fritz found 0.25‰/% for eight North American sites. Lipp *et al.* (1996) found a response of 0.14‰/% for cellulose of *Tamarix jordanis* from nine sites in Israel. The reduced humidity signal in the latter case was attributed to evaporation-limiting adaptations by the trees which allow them to survive in arid environments, such as excretion of a salt crust on the leaves.

A humidity response of less than 0.36‰/% of RH may also result from damping by the so-called *f* factor (Allison *et al.*, 1985; Saurer *et al.*, 1997; and Anderson *et al.*, 1998). This is the percentage of leaf water not subject to evaporative enrichment due to heterogeneity of water in the leaves (Yakir *et al.*, 1994). The mixing of enriched and non-enriched water prior to cellulose synthesis would reduce the humidity signal apparent in cellulose (Anderson *et al.*, 1998). Based on analyses of Swiss conifers, Saurer *et al.* (1997) estimated *f* at 0.5 to 0.7 (50% to 70%) while Anderson *et al.* (1998) found *f* = 0.4 produced better results, although this did not account for local hydrologic effects, since neither soil nor stem water were sampled.

What is remarkable is that a $\Delta^{18}\text{O}$ response of approximately 0.3‰/% of RH can be found in both cellulose and chitin. This implies that the effects of evapotranspirative enrichment on plant leaf water and the carbohydrates synthesized from it are passed directly on to the chitin produced by insects feeding on those carbohydrates. The robustness of this humidity-dependent signal contradicts findings by DeNiro and Cooper (1982). In experiments with potatoes they found that any humidity-dependent signal incorporated in starch synthesized in the leaves was reset by stem water during cellulose synthesis. This implies that the $\delta^{18}\text{O}$ signature of wood cellulose should preserve no humidity-

related effects. However, the results of this study suggest that this is not the case and that no such complete resetting of the $\delta^{18}\text{O}$ signal occurs during chitin synthesis either.

The foregoing suggests that the isotopic separation between $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ is fixed at a $\Delta^{18}\text{O}_{\text{cellulose-chitin}}$ value of approximately 3.5‰, which would appear to be attributable to differences in the biological fractionation of glucose during the synthesis of chitin and cellulose. This is supported by a plot of $\delta^{18}\text{O}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{cellulose}}$ (Figure 4.8): While a linear regression indicates that there is not a one-to-one relationship between these two values, a line (dashed in Figure 4.8) with a slope of one and a fixed depletion of $\delta^{18}\text{O}_{\text{chitin}}$ with respect to $\delta^{18}\text{O}_{\text{cellulose}}$ of 3.50‰ is also a good fit. Furthermore, when the data for sites 1 and 6 (indicated by arrows in Figure 4.8) are removed the best-fit line becomes $y = 1.01x - 3.69$, $r^2 = 0.84$.

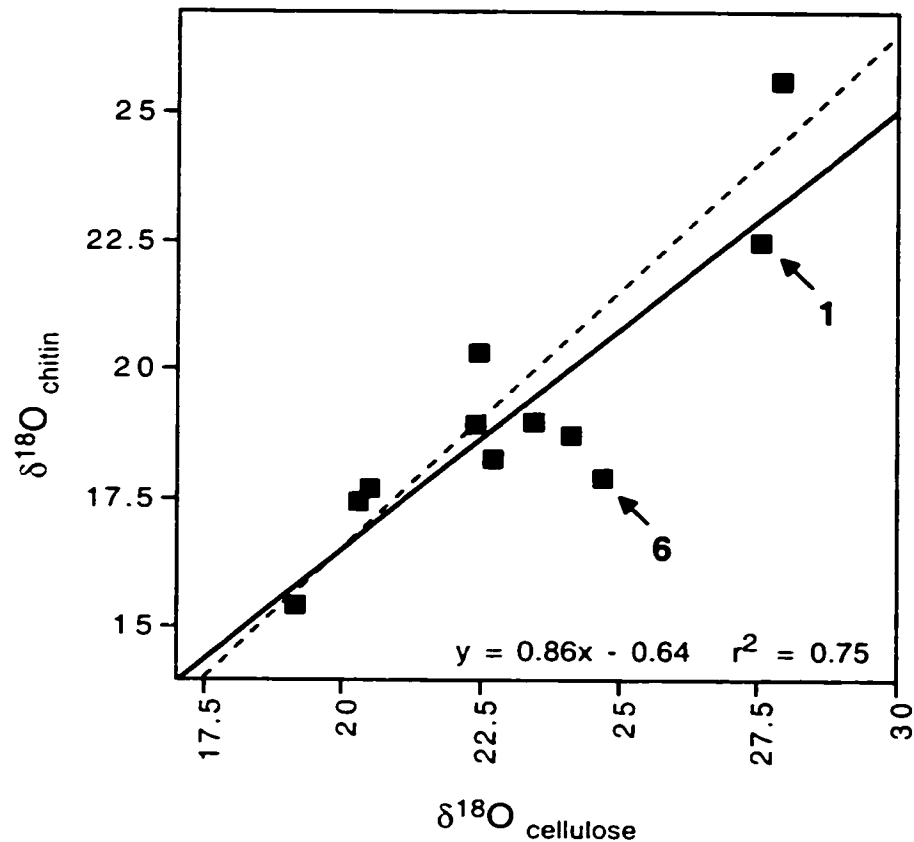


Figure 4.8: $\delta^{18}\text{O}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{cellulose}}$ with the best fit (solid) line and regression. The dashed line represents a one-to-one relationship with a fixed chitin depletion of 3.50‰ so that $y = x - 3.50$. When sites one and six (indicated by arrows) are excluded, the best fit line is $y = 1.01x - 3.69$, $r^2 = 0.84$.

Based on this separation between chitin and cellulose, it is possible to modify the Edwards and Fritz (1986) model to relate the total fractionation between chitin and environmental water to the various fractionation factors which affect the oxygen in the plants and insects. The original model was developed to describe a similar

relationship between cellulose and environmental water and is expressed:

$$\frac{1000 + \delta^{18}\text{O}_{\text{cellulose}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}} = {}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k - {}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)h \quad (4.1)$$

Where $\frac{1000 + \delta^{18}\text{O}_{\text{cellulose}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}}$ is the total fractionation factor,

${}^{18}\alpha_{\text{cellulose-water}}$, ${}^{18}\alpha_n$ is the net biochemical fractionation factor for oxygen during cellulose synthesis and ${}^{18}\alpha_e$ and ${}^{18}\alpha_k$ are the equilibrium and kinetic fractionation factors for oxygen, between the liquid and vapour phases of water and h is the relative humidity. By assuming fixed fractionation factors, this equation relates variations in ${}^{18}\alpha_{\text{cellulose-water}}$ entirely to changes in relative humidity. Edwards and Fritz (1986) used the values ${}^{18}\alpha_n = 1.0282$, ${}^{18}\alpha_e = 1.0095$ and ${}^{18}\alpha_k = 1.0160$ for their calculations. In a survey of other studies, Buhay, *et al.* (1996) found a range of ${}^{18}\alpha_k$ values varying from about 1.0100 to 1.0320 with a mean of 1.0184 ± 0.0061 , depending on leaf morphology and wind speed.

In order to apply this model to the chitin analyses from the present study the ${}^{18}\alpha_n$ must be adjusted to reflect the approximately 3.5‰ depletion of chitin with respect to cellulose. Using $\delta^{18}\text{O}_{\text{chitin}} = 18.95\text{‰}$ and $\delta^{18}\text{O}_{\text{cellulose}} = 22.42\text{‰}$ values from site 8, which fall on the $y = 1.01x - 3.69$ line from Figure 4.8, produces an ${}^{18}\alpha_{\text{chitin-cellulose}}$ of 0.9966, which is multiplied by the ${}^{18}\alpha_n$ for cellulose of 1.0282 to produce an ${}^{18}\alpha_n$ for chitin of 1.0247. Intriguingly, this figure is quite close to the ${}^{18}\alpha_{\text{cellulose-water}}$ of 1.0240 for nonaquatic and semiaquatic arthropods suggested by the data in Schimmelmann and DeNiro (1986a). Although their data show considerable scatter, this ${}^{18}\alpha_{\text{cellulose-water}}$ figure seems to indicate that there was little evaporation-related enrichment exhibited by their samples.

All the host trees in this study have needle-type leaves (dissected morphology) which have higher $^{18}\alpha_k$ values, approximately in a range from 1.0220 to 1.0300 (Buhay, *et al.*, 1996). Therefore:

$$\frac{1000 + \delta^{18}\text{O}_{\text{chitin}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}} = {}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k - {}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)h \quad (4.2)$$

Where ${}^{18}\alpha_n = 1.0247$, ${}^{18}\alpha_e = 1.0095$ and ${}^{18}\alpha_k = 1.0220$ to 1.0300 .

This expression is a linear relationship where $x =$ relative humidity, $y = {}^{18}\alpha_{\text{chitin-environmental water}}$, ${}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)$ is the slope and ${}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k$ is the intercept. This can be used to calculate ${}^{18}\alpha_{\text{chitin-environmental water}}$ from a variety of ${}^{18}\alpha_k$ values (Figure 4.9). Based on this exercise, ${}^{18}\alpha_k = 1.0260$ provides the best-fit line for the sites under discussion. A plot of actual ${}^{18}\alpha_{\text{chitin-environmental water}}$ (Figure 4.10) validates the model results with a linear fit (line A) which is close to the line generated with calculated values (line B) when ${}^{18}\alpha_k = 1.0260$. The lower slope of line A is apparently due to the influence of the data from Kelsey Bay, B. C. (site 14), which has a considerable affect on the slope. Plotting calculated against actual ${}^{18}\alpha_{\text{chitin-environmental water}}$ values (Figure 4.11) further confirms the choice of ${}^{18}\alpha$ values and the model results by producing a linear fit close to $y = x$. The fact that theoretical and actual ${}^{18}\alpha_{\text{chitin-environmental water}}$ values have the same relationship with humidity, and their one-to-one correspondence, suggests that the f factor is not damping the humidity signal in chitin. If the f factor were significant the slope of the actual ${}^{18}\alpha_{\text{chitin-environmental water}}$ versus humidity line would be lower than the slope of the line constructed from calculated values and the calculated ${}^{18}\alpha_{\text{chitin-environmental water}}$ versus actual ${}^{18}\alpha_{\text{chitin-environmental water}}$ line would have a slope greater than one. The absence of an f factor effect on $\delta^{18}\text{O}_{\text{chitin}}$ suggests that the insects are feeding on sugars that have not exchanged with non-evaporatively enriched oxygen or that the sites in this study are, for some reason, not subject to the f factor.

Therefore, the Edwards and Fritz (1986) model, with fractionation factors selected to suit chitin and the food plants of the insects, provides a reasonable description of the modification of the isotopic composition of oxygen as it progresses from environmental water to chitin.

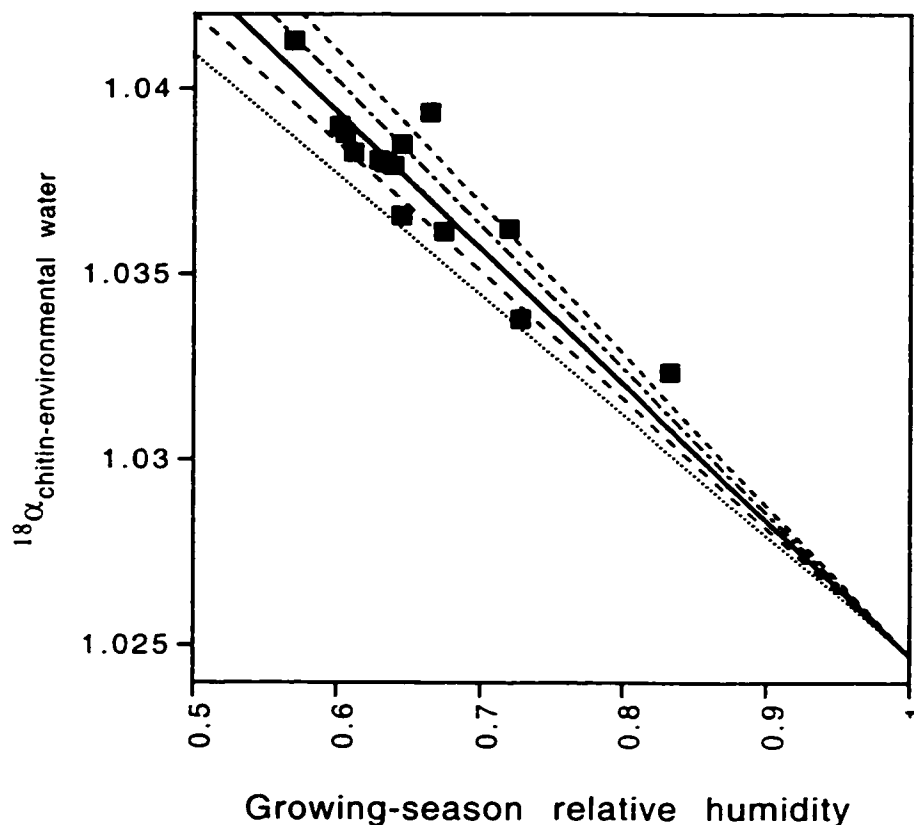


Figure 4.9: Calculated $^{18}\alpha_{\text{chitin-environmental water}}$ versus growing-season relative humidity for various $^{18}\alpha_k$ values. The lines are, from top to bottom, $^{18}\alpha_k = 1.0300$, $^{18}\alpha_k = 1.0280$, $^{18}\alpha_k = 1.0260$, $^{18}\alpha_k = 1.0240$ and $^{18}\alpha_k = 1.0220$. The points are the actual $^{18}\alpha_{\text{chitin-environmental water}}$ values for the various sites. A value of $^{18}\alpha_k = 1.0260$ provides the best fit for the data.

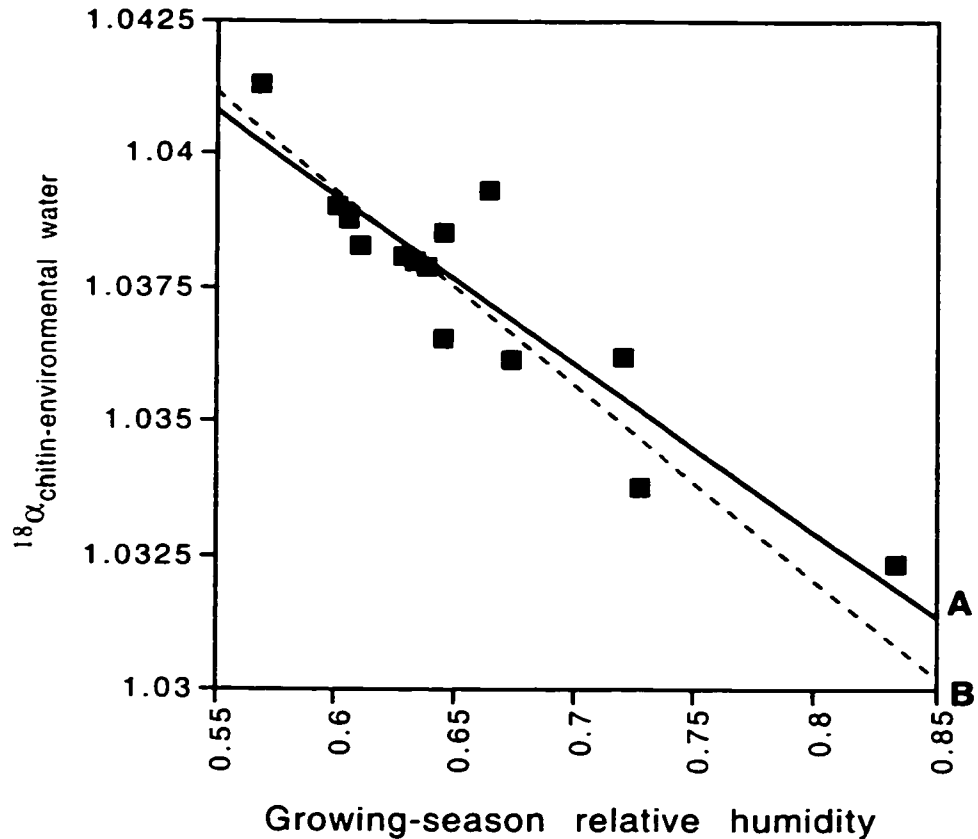


Figure 4.10: Actual $^{18}\alpha_{\text{chitin-environmental water}}$ versus growing-season relative humidity. Line A is a linear regression through the data points, line B is for calculated $^{18}\alpha_{\text{chitin-environmental water}}$ (from Figure 4.9) using $^{18}\alpha_k = 1.0260$. This shows that true and theoretical values for $^{18}\alpha_{\text{chitin-environmental water}}$ are in good agreement.

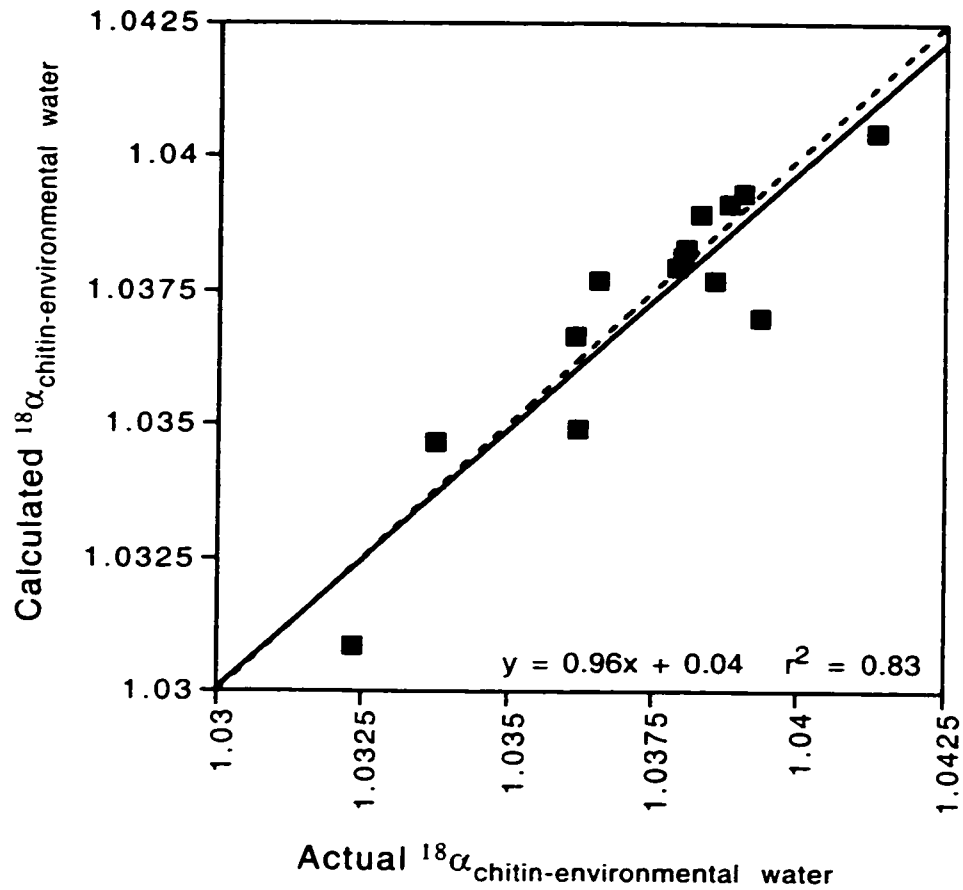


Figure 4.11: Calculated $^{18}\alpha_{\text{chitin-environmental water}}$ versus actual $^{18}\alpha_{\text{chitin-environmental water}}$ values, demonstrating the excellent correlation between true and theoretical $^{18}\alpha_{\text{chitin-environmental water}}$. The dashed line is $y = x$, showing that the actual and calculated values approach a one-to-one relationship.

Plotting $\delta^{18}\text{O}_{\text{chitin}}$ normalized for 100% RH, based on a humidity response of $-0.29\text{‰}/\%$ RH from Figure 4.6, against $\delta^{18}\text{O}_{\text{environmental water}}$ (Figure 4.12) vastly improves the linear correlation between the two sets of values (compared to Figure 4.4), confirming that variations in evaporative enrichment between the sites is responsible for much of the scatter in Figure 4.4. Values for $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{environmental water}}$ on the regression line in Figure 4.12 (e.g. site 11: $\delta^{18}\text{O}_{\text{chitin}}$ (normalized) = 6.62‰ ; $\delta^{18}\text{O}_{\text{environmental water}}$ = -19.8‰) produce $^{18}\alpha_{\text{chitin-environmental water}}$ values of approximately 1.0270, which is higher than the 1.0247 expected under conditions of zero evaporative enrichment. This discrepancy may result from the $-0.29\text{‰}/\%$ of RH correction undercompensating for the effects of evaporation.

Figure 4.13 shows the relationship between $\delta^{18}\text{O}_{\text{chitin}}$ and annual temperature. It reflects the $\delta^{18}\text{O}_{\text{environmental water}}$ versus annual temperature relationship, which has a linear correlation of $\delta^{18}\text{O}_{\text{environmental water}} = 0.85 \text{ T}^\circ\text{C} - 18.37$, $r^2 = 0.68$. The principal differences between the two regressions are a higher slope for the $\delta^{18}\text{O}_{\text{environmental water}}$ versus temperature relationship and a relative enrichment in the chitin values. The difference in slopes reflects a general trend toward increasing RH with increasing annual temperature. Since increasing temperature increases $\delta^{18}\text{O}_{\text{chitin}}$, through the $\delta^{18}\text{O}_{\text{environmental water}}$ -temperature relationship, while increasing RH lowers $\delta^{18}\text{O}_{\text{chitin}}$, through decreased evapotranspirative enrichment of food-plant material, the temperature and humidity effects would, to some extent, cancel each other out, resulting in a lower slope in the $\delta^{18}\text{O}_{\text{chitin}}$ versus temperature relationship.

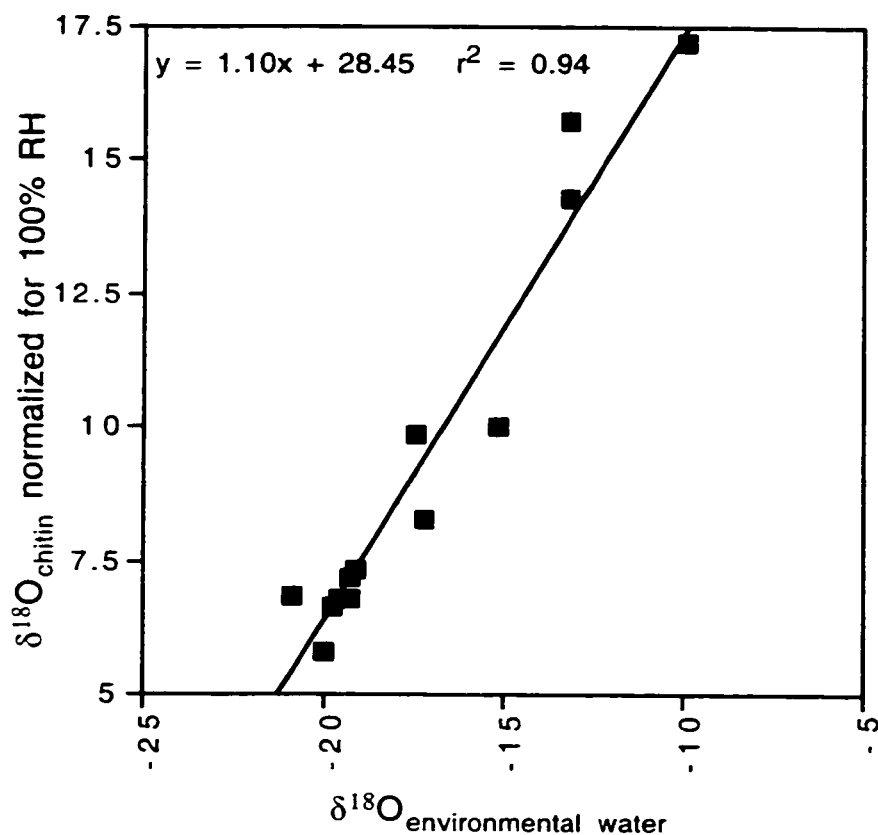


Figure 4.12: $\delta^{18}\text{O}_{\text{chitin}}$ normalized for 100% RH versus $\delta^{18}\text{O}_{\text{environmental water}}$. $\delta^{18}\text{O}_{\text{chitin}}$ is normalized based on -0.29‰/‰ of RH from Figure 4.6. This shows that removing the humidity-related variability in this relationship results in an improved linear fit.

Normalized for 100% RH (Figure 4.14) the linear fit is improved and the slope of 0.96 becomes much closer to that of the $\delta^{18}\text{O}_{\text{environmental-water}}$ versus annual temperature relationship. Also, the difference in intercepts of 26.53 is very similar to the average enrichment of aquatic chitin over water of 26‰ observed by Schimmelmann and DeNiro (1986a).

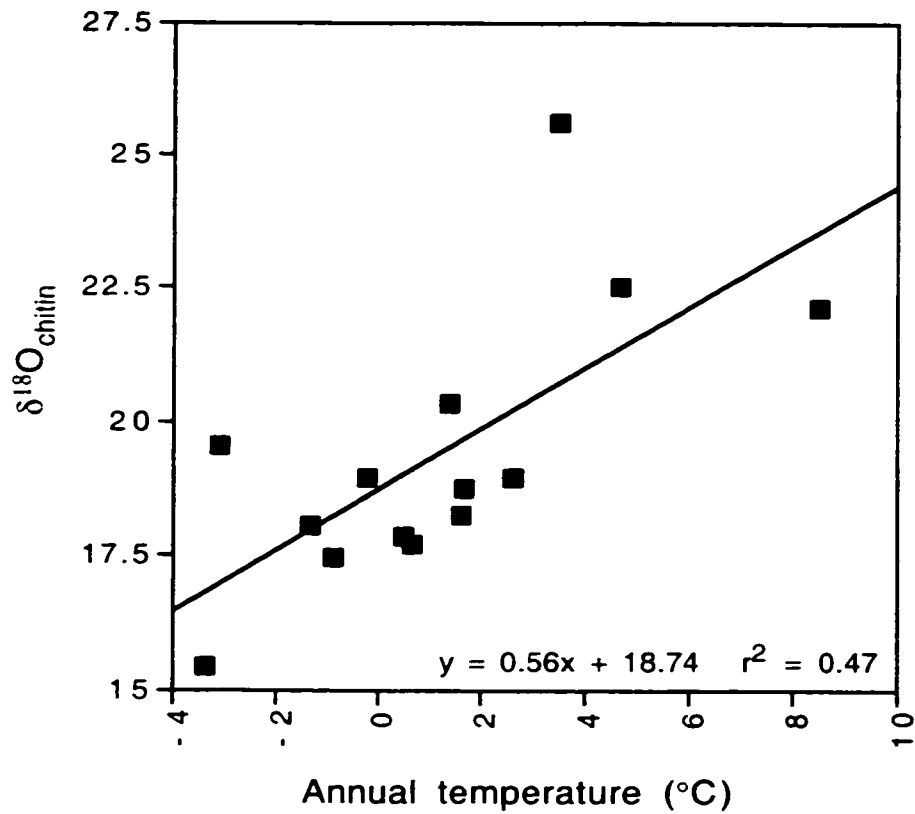


Figure 4.13: $\delta^{18}\text{O}_{\text{chitin}}$ versus annual temperature, which reflects the $\delta^{18}\text{O}_{\text{environmental water}}$ versus annual temperature relationship of $\delta^{18}\text{O}_{\text{environmental water}} = 0.85 \text{ T}^\circ\text{C} - 18.37$, $r^2 = 0.68$, modified by the effects of varying relative humidity and biological processes.

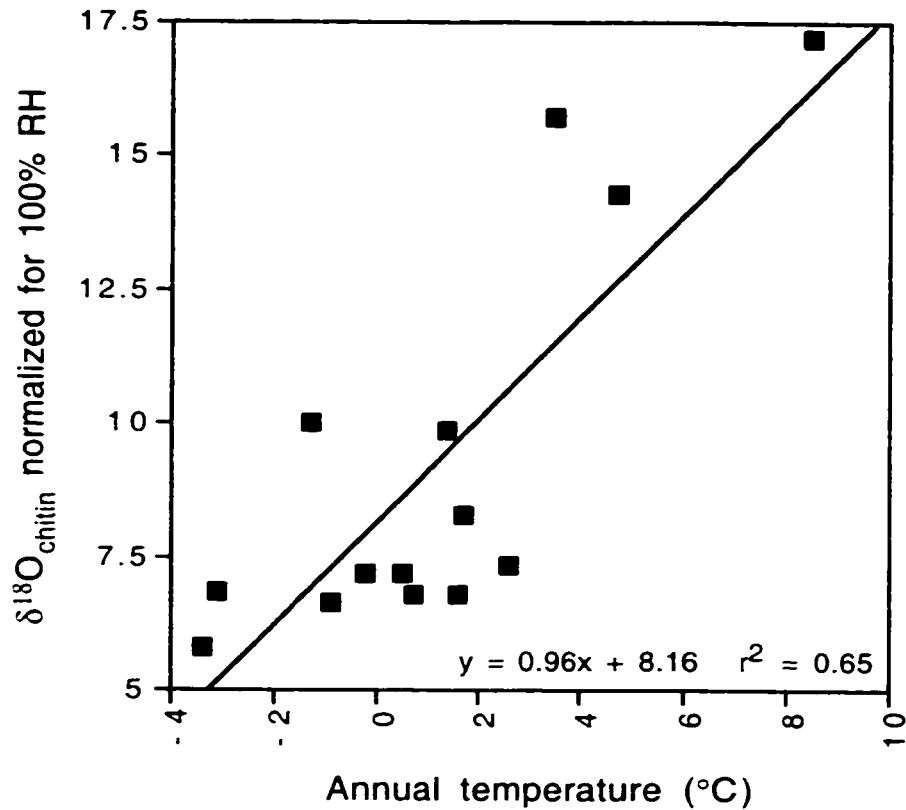


Figure 4.14: $\delta^{18}\text{O}_{\text{chitin}}$ normalized for 100% RH versus annual temperature. $\delta^{18}\text{O}_{\text{chitin}}$ is normalized based on $-0.29\text{‰}/\%$ of RH from Figure 4.6. Normalization removes the effects of varying relative humidity, improving the linear correlation and bringing the regression closer to the $\delta^{18}\text{O}_{\text{environmental water}}$ versus annual temperature relationship of $\delta^{18}\text{O}_{\text{environmental water}} = 0.85 T^{\circ}\text{C} - 18.37$. Enrichment resulting from biological processes may cause the difference in intercepts between the two equations.

4.3: Hydrogen discussion:

Figure 4.15 shows the relationships between the $\delta^2\text{H}$ values for cellulose, chitin and environmental water for each site. The average enrichment of chitin with respect to environmental water is $38.5 \pm 25.2\text{‰}$, while the average enrichment over cellulose is $53.5 \pm 15.6\text{‰}$. It is clear that, while there is a general correlation among the trends in $\delta^2\text{H}$ for the three materials, there is considerable variability, to the point where there is an anti-correlative relationship between chitin and cellulose for many sites. In comparison, data in Miller, *et al.* (1988) show an average $\delta^2\text{H}$ enrichment for chitin over environmental water of $13.9 \pm 26.1\text{‰}$ for beetles from 32 sites across North America. Experiments by Miller (1984) suggested an 8-11‰ enrichment over plant material. Schimmelmann and DeNiro (1986a) found enrichments over environmental water of 10 to 95‰ for marine arthropod chitin.

Site Number	Site Name	Taxon	Diet
1	West Bay, Ont.	<i>Ips pini</i>	wood
2	Espanola, Ont.	<i>Monochamus scutellatus</i>	wood
3	Moosonee, Ont.	<i>Phaedon</i> sp.	plants
4	Pine River, Man.	<i>Dendroctonus rufipennis</i>	wood
5	Swan River, Man.	<i>Ips pini</i>	wood
6	Prince Albert, Sask.	<i>Ips pini</i>	wood
7	Glaslyn, Sask.	<i>Ips pini</i>	wood
8	Elk Isl. Nat. Park, Alta.	<i>Ips pini</i>	wood
9	Grand Prairie, Alta.	<i>Ips pini</i>	wood
10	64 km. N. of Manning, Alta.	<i>Polygraphus rufipennis</i>	wood
11	High Level, Alta.	<i>Ips latidens</i>	wood
12	Hay River, N.W.T.	<i>Polygraphus rufipennis</i>	wood
13	Watson Lake, Y.T.	<i>Arhopalus foveicollis</i>	wood
14	Kelsey Bay, B.C.	<i>Tripodendron lineatum</i>	fungus

Table 4.3: Sites and taxa used for $\delta^2\text{H}$ analyses.

Site Number	Chitin $\delta^2\text{H}$ (‰ \pm 3.4)	Cellulose $\delta^2\text{H}$ (‰ \pm 4.1)	Water $\delta^2\text{H}$ (‰)	Annual Temperature (°C \pm 1)	Growing-Season Relative Humidity (%)
1	-90.1	-144.7	-94	4.7	72 \pm 4
2	-96.4	-138.9	-94	3.5	66 \pm 6
3	-115.0		-112	-1.3	73 \pm 4 *
4	-86.3	-134.4	-129	1.7	64 \pm 5
5	-94.8	-128.9	-134	1.4	64 \pm 5
6	-79.5	-140.8	-147	0.5	64 \pm 6
7	-86.0	-147.6	-147	-0.2	60 \pm 5
8	-84.1	-131.1	-149	2.6	61 \pm 7
9	-113.7	-139.3	-152	1.6	61 \pm 4 *
10	-93.0	-162.9	-157	0.7	63 \pm 4 *
11	-109.4	-177.1	-159	-0.9	63 \pm 4 *
12	-100.9	-176.7	-162	-3.4	67 \pm 3 *
13	-130.0		-163	-3.1	57 \pm 5 *
14	-51.0		-71	8.5	83 \pm 3

Table 4.4: RH values are averages for 0400 to 1900 local time for May through September and June through August (*). Precision for water $\delta^2\text{H}$ is estimated to be on the order of \pm 4.0‰.

Site	Site name	Chitin $\delta^2\text{H}$ (‰ \pm 4)	Water $\delta^2\text{H}$ (‰)	Annual Temperature (°C \pm 1)	Growing-Season Relative Humidity (%)
15	Tuktoyaktuk, N.W.T.	-90	-160	-10.7	79 \pm 2 *
16	Canoe L., N.W.T.	-104	-160	-10.7	79 \pm 2 *
17	Involuted Hills, N.W.T.	-99	-160	-10.7	79 \pm 2 *
18	Whitehorse, Y.T.	-110	-158	-1.1	60 \pm 5 *
19	North Kluane, Y.T.	-104	-140	-2.7	66 \pm 4 *
20	Fairbanks, AK	-105	-150	-3.4	58 \pm 8 *
21	Bettles, AK	-102	-150	-7.0	75 \pm 5 *
22	Umiat, AK	-97	-127	-9.5	81 \pm 2 *
23	Okpikruak R., AK	-114	-127	-9.0	81 \pm 2 *
24	Yellowknife, N.W.T.	-115	-146	-5.3	61 \pm 6 *
25	Rankin Inlet, N.W.T.	-78	-130	-11.6	79 \pm 2 *
26	Churchill, Man.	-100	-120	-7.4	78 \pm 1 *
27	Seattle, WA	-78	-90	11.7	68 \pm 3
28	Burnaby, B.C.	-88	-100	9.1	75 \pm 3
29	Queen Charlotte Is., B.C.	-80	-70	7.6	81 \pm 1
30	Niwot Ridge, CO	-78	-80	10.0	47 \pm 1
31	Gila Bend, AZ	-76	-67	21.3	36 \pm 6 ***
32	Amidon, ND	-93	-90	5.6	64 \pm 3
33	Rushing R. Pr. Pk., Ont.	-99	-90	2.0	67 \pm 5
34	Sault Ste. Marie, Ont.	-82	-80	5.0	75 \pm 4
35	Timmins, Ont.	-89	-90	1.1	71 \pm 5
36	Hailebury, Ont.	-78	-80	3.7	71 \pm 5
37	Waterloo, Ont.	-58	-70	7.3	73 \pm 3
38	Prince Edward Is.	-65	-70	5.8	77 \pm 2
39	Indianapolis, IN	-54	-50	10.6	69 \pm 3
40	Caryville, TN	-55	-50	15.1	69 \pm 5 **
41	Charleston, SC	-49	-25	18.3	74 \pm 4 **
42	Waycross, GA	-42	-25	20.1	72 \pm 5 **
43	Valdosta, GA	-26	-25	20.1	72 \pm 5 **
44	Venice, FL	-47	-25	22.3	71 \pm 4 ***
45	Kentwood, LA	-13	-24	18.9	74 \pm 3 ***
46	Espey, TX	-40	-24	19.6	73 \pm 2 ***

Table 4.5: Data from Miller *et al.* (1988). RH values are averages for 0400 to 1900 local time for May through September and June through August (*), April through October (), and the whole year (***). Water $\delta^2\text{H}$ precision is estimated to be on the order of $\pm 4.0\%$.**

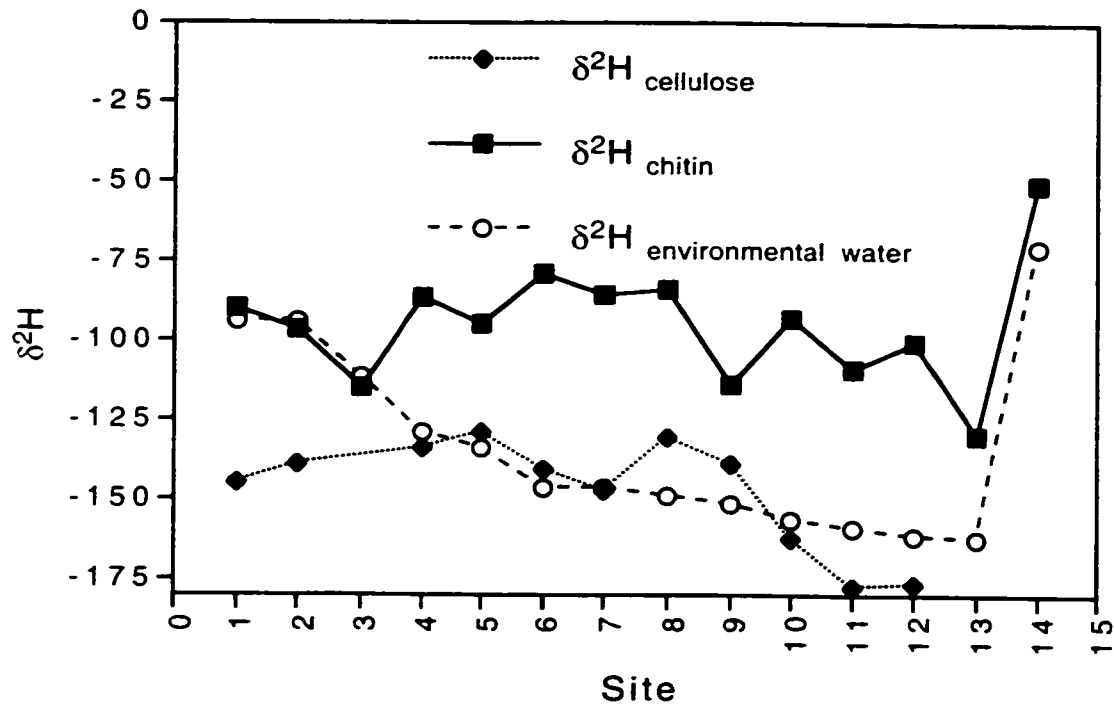


Figure 4.15: $\delta^2\text{H}$ of cellulose, chitin and environmental water versus site for this study. The roughly parallel trends suggest that environmental water isotopic composition is influencing those of chitin and cellulose.

Figure 4.16 plots the $\delta^2\text{H}$ data for sites from this study and Miller, *et al.* (1988). Once again, while the general trends of $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{environmental water}}$ are similar, there is significant variability. This is particularly evident in sites 4 to 26, where $\delta^2\text{H}_{\text{chitin}}$ shows considerable enrichment over $\delta^2\text{H}_{\text{environmental water}}$. Plotting $\delta^2\text{H}_{\text{chitin}}$ versus $\delta^2\text{H}_{\text{environmental water}}$ (Figures 4.17 and 4.18) shows that, while there is a linear relationship between these variables, it is being skewed away from a slope of one. The correlation is better than that observed by Schimmelmann and DeNiro (1986a), who found linear regressions produced correlation coefficients close to zero for semi- and non-aquatic arthropods.

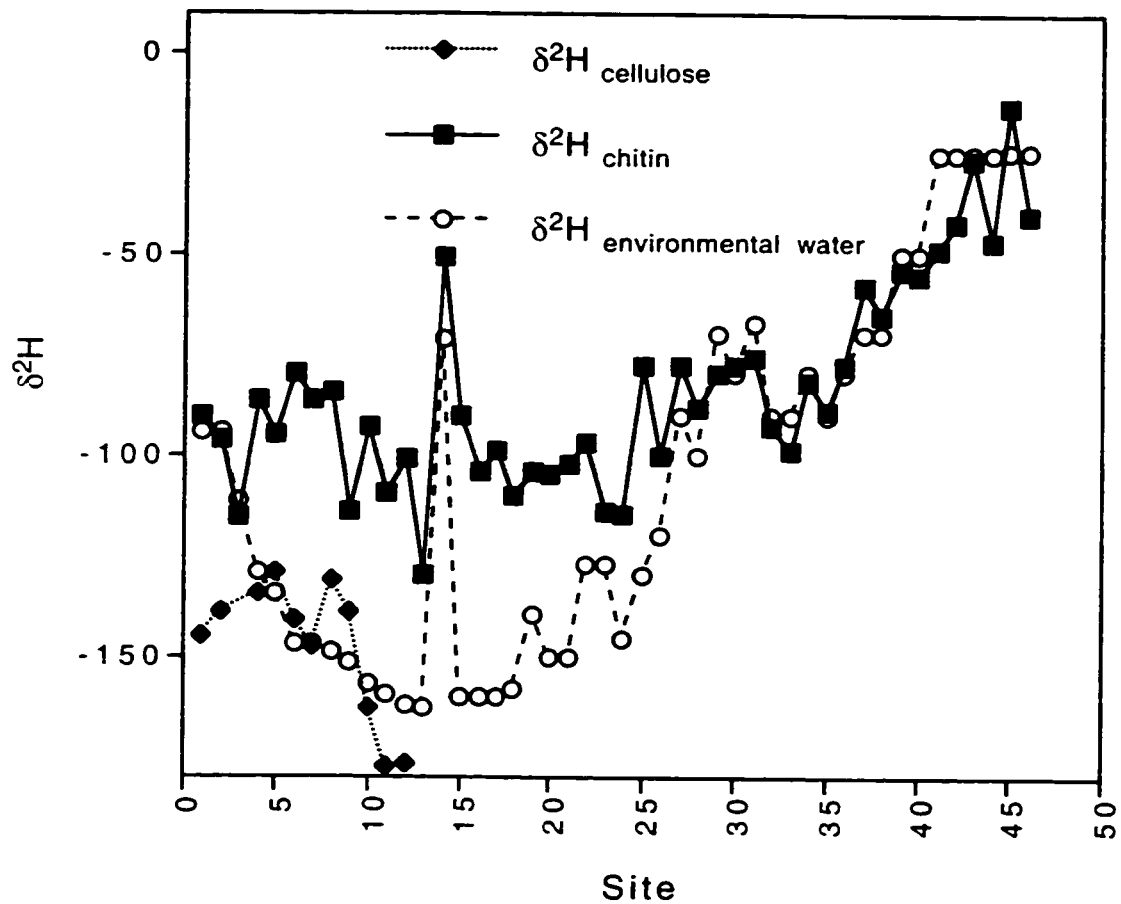
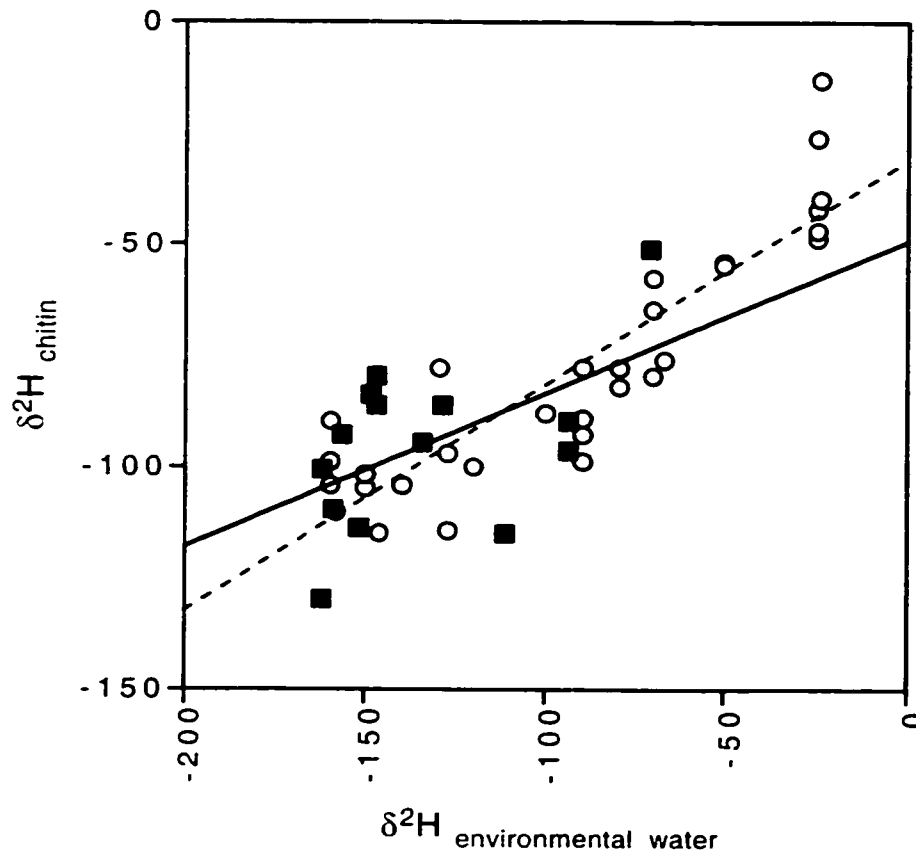


Figure 4.16: $\delta^{2}\text{H}$ of cellulose, chitin and environmental water versus site using data from this study and Miller *et al.* (1988). As in Figure 4.15, roughly parallel trends show that environmental water $\delta^{2}\text{H}$ is influencing the isotopic content of chitin. Note the enrichment of $\delta^{2}\text{H}_{\text{chitin}}$ over $\delta^{2}\text{H}_{\text{environmental water}}$ in sites 4 to 26, possibly because of preferential exposure of the insects to relatively enriched summer precipitation due to seasonal activity in cooler locations (see discussion in text).



- This study: $y = 0.34x - 49.43$ $r^2 = 0.28$
- Miller *et al.* (1988): $y = 0.50x - 31.77$ $r^2 = 0.80$

Figure 4.17: $\delta^{2}\text{H}_{\text{chitin}}$ versus $\delta^{2}\text{H}_{\text{environmental water}}$ showing data from this study (solid line) and Miller *et al.* (1988) (dashed line). Note that the two data sets appear to be well integrated despite differences in the time of sample collection and analytical methodology.

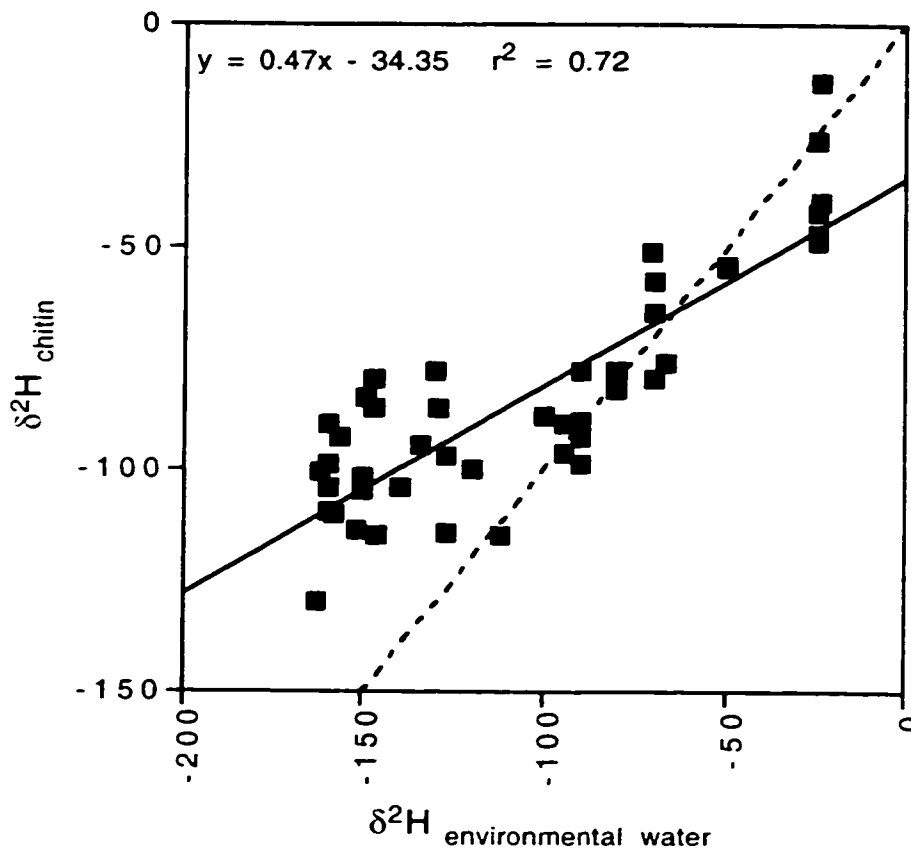


Figure 4.18: Regression using the entire data set from Figure 4.17. Dashed line is $y = x$. The relationship is skewed away from a slope of one, showing that unresolved fractionating effects are present.

Edwards and Fritz (1986) found there is a correlation between relative humidity and the apparent fractionation between $\delta^{2}\text{H}_{\text{environmental water}}$ and $\delta^{2}\text{H}_{\text{cellulose}}$. Yapp and Epstein (1982b) concluded that relative humidity did have an effect on the apparent fractionation between $\delta^{2}\text{H}_{\text{cellulose}}$ and $\delta^{2}\text{H}_{\text{environmental water}}$. However, Terwilliger and DeNiro (1995) found, in experiments with avocado seedlings, that wood cellulose $\delta^{2}\text{H}$ did not reflect variations in atmospheric relative humidity, presumably because of the influence of non-evapotranspiratively enriched stem water on cellulose synthesis in the plants' woody tissues. In a study of white pine

in southeastern New York State which incorporated all variables thought to affect $\delta^{2}\text{H}_{\text{cellulose}}$. White *et al.* (1994) speculated that mutually-canceling atmospheric and evaporative effects erased the humidity-dependent signature from cellulose. They concluded, however, that these effects may not counteract each other in all environments.

Chitin may inherit any $\delta^{2}\text{H}$ -humidity signal from cellulose precursors, as was the case with oxygen. To test whether any humidity-related effects are evident in chitin, $\Delta^{2}\text{H}_{\text{chitin-environmental water}}$ ($\delta^{2}\text{H}_{\text{chitin}} - \delta^{2}\text{H}_{\text{environmental water}}$) versus RH was plotted for all 46 sites (Figure 4.19). There is no humidity-dependent signal distinguishable in $\delta^{2}\text{H}_{\text{chitin}}$. It is not clear whether this is due to equilibration with unenriched water in either the food plant or the insect (Terwilliger and DeNiro, 1995), or whether it is being obscured by some other effect.

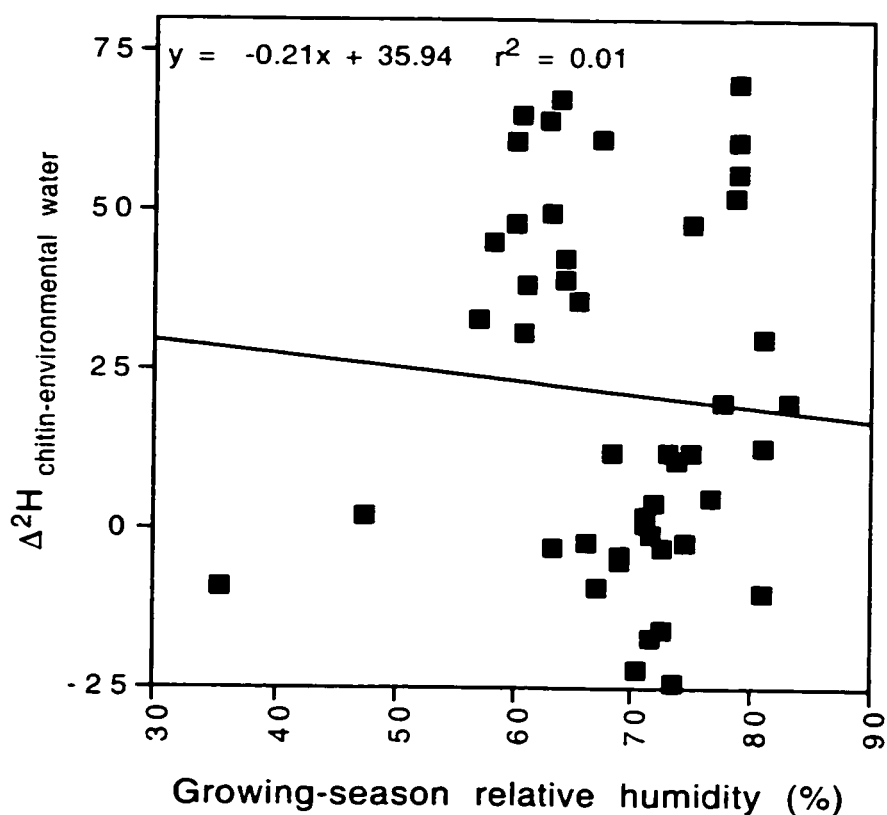


Figure 4.19: $\Delta^{2}\text{H}_{\text{chitin-environmental water}}$ versus RH. Regression is for all sites. There appears to be no relationship between relative humidity and enrichment of chitin over environmental water.

Miller, *et al.* (1988) found that $\delta^2\text{H}_{\text{chitin}}$ became more enriched with respect to environmental water at colder sites. This is apparent in Figure 4.16 where $\delta^2\text{H}_{\text{chitin}}$ is significantly enriched over $\delta^2\text{H}_{\text{environmental water}}$ in relatively-cold sites 4 to 26. That study suggested that this effect may reflect that insects are only active in warmer months, and the short season in cold areas may cause them to be selectively exposed to relatively-enriched summer precipitation, in an effect analogous to snowmelt bypass in lakes. It is also suspected that temperature has an influence on fractionation of $\delta^2\text{H}$ (White *et al.*, 1994; Buhay *et al.*, 1996) of about $1.3\text{‰}/^\circ\text{C}$, occurring prior to cellulose synthesis (Buhay *et al.*, 1996), although this is balanced by an opposing effect of $-1.1\text{‰}/^\circ\text{C}$ on the equilibrium fractionation of hydrogen between water in liquid and vapour states (White *et al.*, 1994). Figure 4.20 shows that $\Delta\delta^2\text{H}_{\text{chitin-environmental water}}$ from this study and Miller, *et al.* (1988) do exhibit an apparent temperature effect of about $-2.1\text{‰}/^\circ\text{C}$.

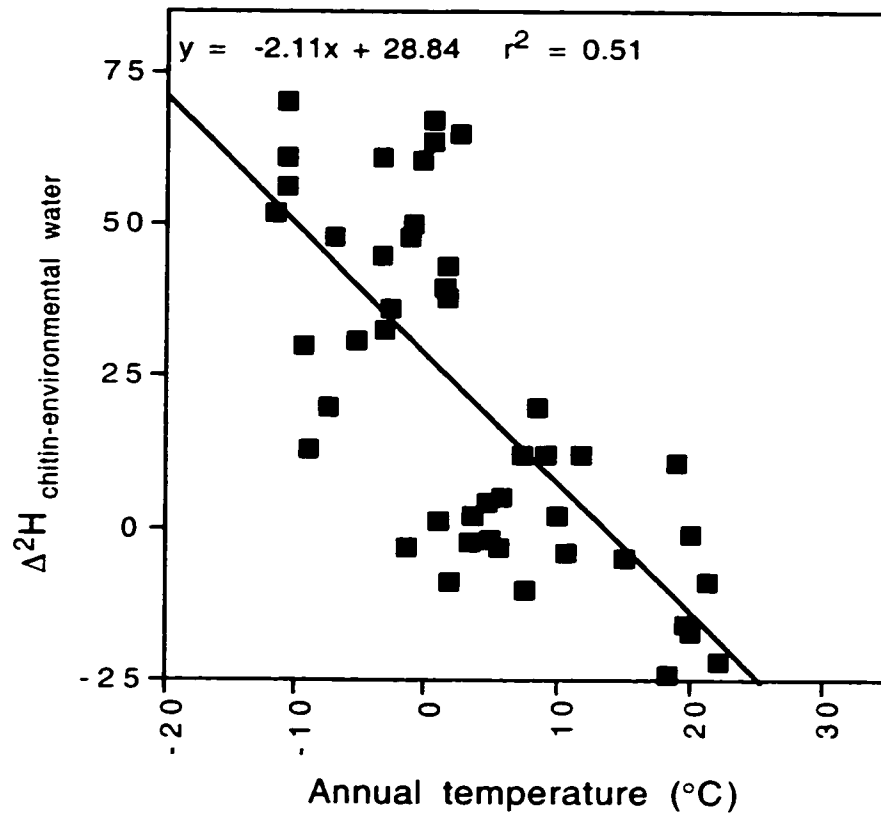


Figure 4.20: $\Delta\delta^2\text{H}_{\text{chitin-environmental water}}$ versus annual temperature, showing that temperature influences the enrichment of chitin over environmental water, with enrichment increasing as annual temperature decreases.

With the knowledge that temperature influences $\delta^2\text{H}_{\text{chitin}}$, it is possible to add this effect to the Edwards and Fritz (1986) model. The model for $\delta^2\text{H}_{\text{chitin}}$ is:

$$\frac{1000 + \delta^2\text{H}_{\text{chitin}}}{1000 + \delta^2\text{H}_{\text{environmental water}}} = {}^2\alpha_n {}^2\alpha_e {}^2\alpha_k - {}^2\alpha_n ({}^2\alpha_e {}^2\alpha_k - 1)h \quad (4.3)$$

Where $\frac{1000 + \delta^2\text{H}_{\text{chitin}}}{1000 + \delta^2\text{H}_{\text{environmental water}}}$ is the total fractionation factor,

${}^2\alpha_{\text{chitin-environmental water}}$, ${}^2\alpha_n$ is the net biochemical fractionation factor for hydrogen during chitin synthesis and ${}^2\alpha_e$ and ${}^2\alpha_k$ are the equilibrium and kinetic fractionation factors for hydrogen, between the liquid and vapour phases of water, and h is the relative humidity. As discussed above, ${}^2\alpha_e$ is temperature-dependent and White *et al.* (1994) inferred a temperature dependence in ${}^2\alpha_n$ for cellulose. Buhay *et al.* (1996) included an apparent temperature component in ${}^2\alpha_k$ for leaf water evaporative enrichment to compensate for a temperature-dependent isotope effect in the leaves, although this was done for computational convenience. However, for the sake of simplicity, this discussion will assume that the temperature effects in cellulose cancel each other out (as in White *et al.* 1994). Therefore, any temperature-related fractionation would be expressed in the ${}^2\alpha_n$ between chitin and water, which will be assumed to be a function of temperature ($^{\circ}\text{C}$). Although this is done for computational convenience, it must be remembered that this effect is not a true fractionation, but instead uses temperature as a proxy for seasonal selection of precipitation. We can solve for this function by rearranging equation 4.3 as follows:

$${}^2\alpha_n = {}^2\alpha_{\text{chitin-environmental water}} / ({}^2\alpha_e {}^2\alpha_k - ({}^2\alpha_e {}^2\alpha_k - 1)h) \quad (4.4)$$

Where h is expressed as a decimal fraction. Since kinetic and equilibrium fractionation of $\delta^2\text{H}$ takes place in the leaves of food plants, ${}^2\alpha_e$ and ${}^2\alpha_k$ for cellulose can be used for modeling chitin. Therefore, ${}^2\alpha_e$ is set at 1.0797 (calculated at 21°C from the equation of Kakiuchi and Matsuo, 1979; Edwards and Fritz, 1986). Values for ${}^2\alpha_k$ are more problematic, since they vary with leaf morphology and wind speed (Buhay *et al.*, 1996). Since these factors are unknown for most of the sites, an average ${}^2\alpha_k$ of 1.0186 from Buhay *et al.* (1996) is used. A regression of a plot of ${}^2\alpha_n$, from equation 4.4, against temperature (Figure 4.21) gives the relationship between these two factors.

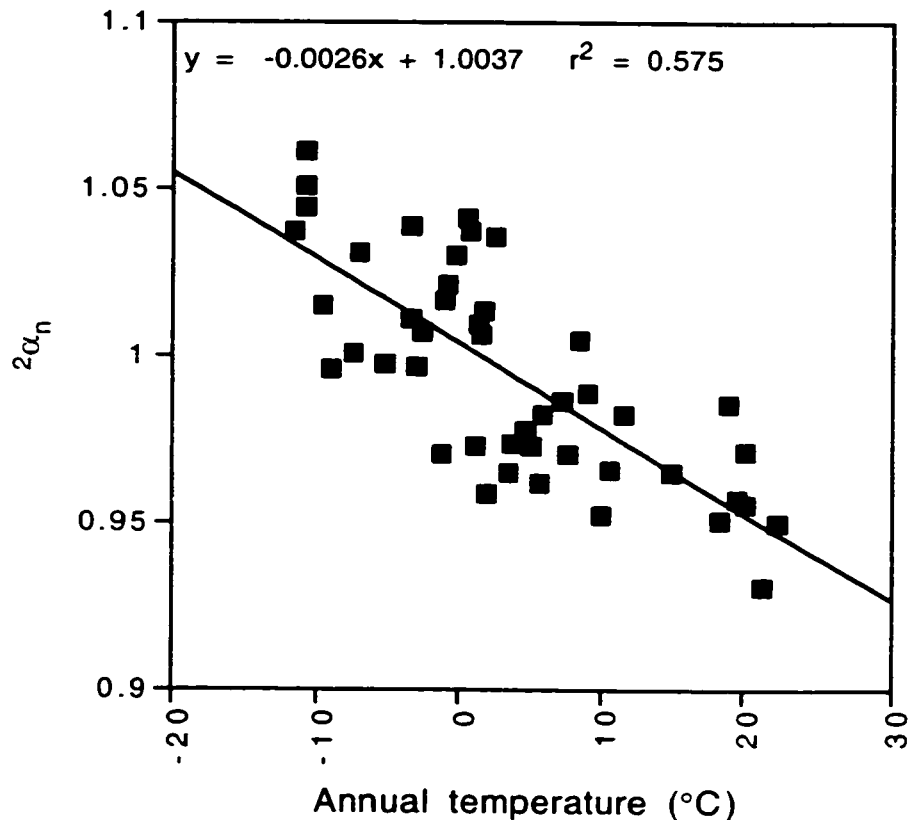


Figure 4.21: ${}^2\alpha_n$ versus temperature. This demonstrates the temperature dependency of the net biochemical fractionation factor for chitin.

It appears in Figure 4.21 that ${}^2\alpha_n$ has a linear relationship with temperature, however, there is some noise in the system. This is no doubt partly due to the simplifying assumptions discussed earlier. While Figure 4.21 and associated calculations assume that ${}^2\alpha_e$ and ${}^2\alpha_k$ are fixed, considerable variability is possible. As noted before ${}^2\alpha_e$ has a temperature component of $-1.1\text{‰}/\text{°C}$ (White *et al.* 1994). Values for ${}^2\alpha_k$ can also vary between 1.0123 and 1.0251 depending on wind speed and leaf morphology (Buhay *et al.* 1996) and the authors included a temperature component of $1.3\text{‰}/\text{°C}$ (to compensate for effects in the leaves), although this is counteracted by the temperature effect on ${}^2\alpha_e$. The ${}^2\alpha_n$ for cellulose (which in this analysis is partially incorporated in

${}^2\alpha_n$ for chitin since chitin is synthesized from photosynthetic products) also shows considerable variability in published reports: Yapp and Epstein (1982b) give values of 0.961 to 0.988 for aquatic plants and suggest a temperature dependence of 2.0‰/°C. Edwards and Fritz (1986) inferred a value of 0.953 for a variety of trees while White *et al.* (1994) inferred a range for white pine from 0.926 to 0.943.

Also, as Yakir (1992) points out, enormous variations can be encountered in the $\delta^2\text{H}$ of plant carbohydrates, depending on where they are sampled, and some of this variation may be passed on to chitin. Furthermore, there is considerable variability and uncertainty in the life habits and foodstuffs of the insects from the various sites and Miller *et al.* (1988) sampled a wide variety of families. Despite these factors, the linear trend in Figure 4.21 is significant.

To test whether the relationship between ${}^2\alpha_n$ and temperature derived above can accurately model the hydrogen isotopic fractionation processes involved in chitin synthesis the figures from the regression in Figure 4.21 are inserted into equation 4.3, along with the previously used values for ${}^2\alpha_e$ and ${}^2\alpha_k$, to produce the expression:

$${}^2\alpha_{\text{chitin-environmental water}} = (-0.0026T+1.0037)(1.0797)(1.0186) - \frac{(-0.0026T+1.0037)((1.0797)(1.0186)-1)h}{(1.0797)(1.0186)-1} \quad (4.5)$$

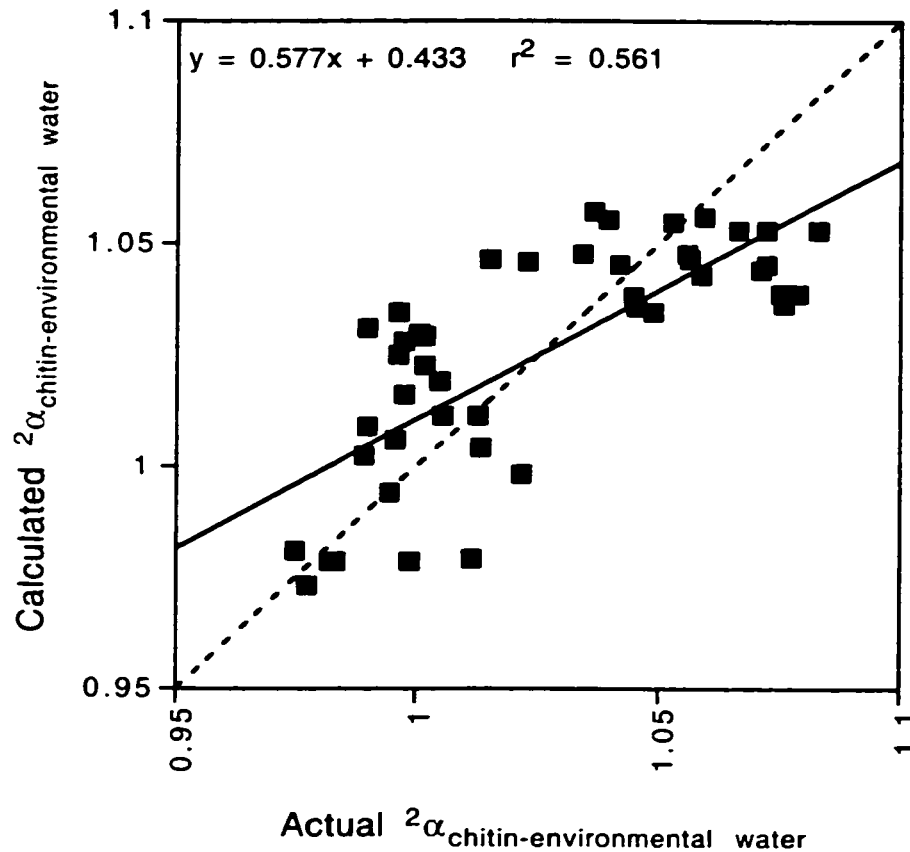
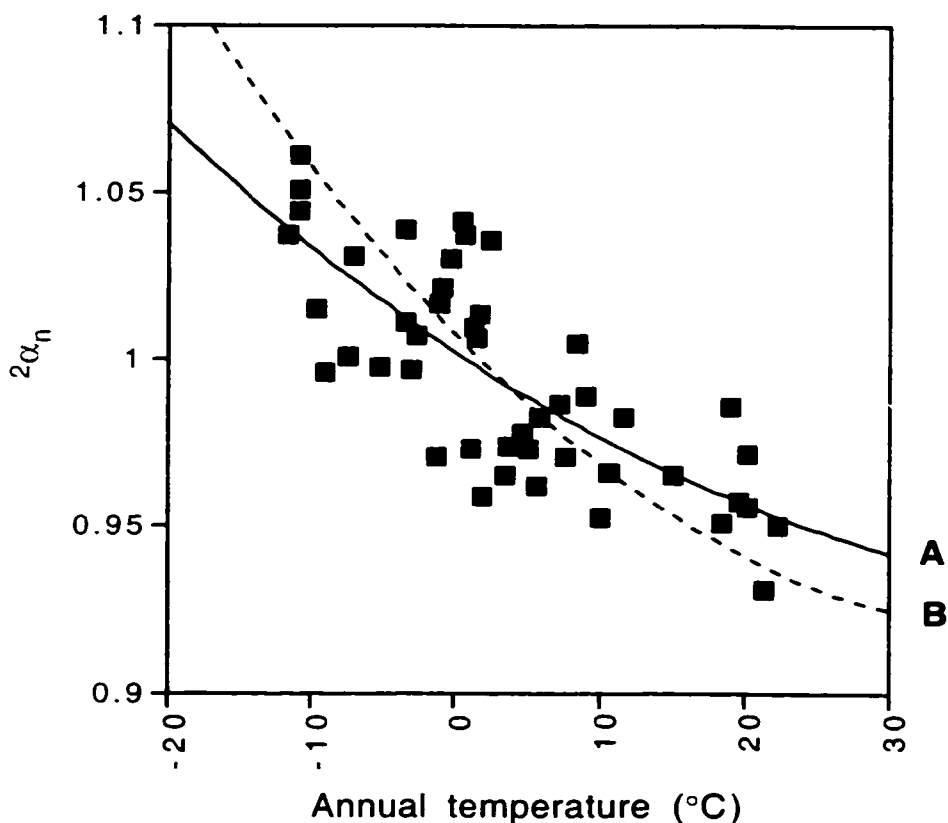


Figure 4.22: ${}^2\alpha_{\text{chitin-environmental water}}$ calculated from equation 4.5 versus actual ${}^2\alpha_{\text{chitin-environmental water}}$. Dashed line is $y = x$. Deviation from a one-to-one relationship shows that the formula for calculating ${}^2\alpha_{\text{chitin-environmental water}}$ is not accurately modeling natural processes.

When plotted (Figure 4.22) against the actual ${}^2\alpha_{\text{chitin-environmental water}}$, the ${}^2\alpha_{\text{chitin-environmental water}}$ calculated from equation 4.5 yields a reasonable linear fit but does not display the expected one-to-one relationship. Examination of Figure 4.22 shows that this is due to underestimation of ${}^2\alpha_{\text{chitin-environmental water}}$ by the model in more enriched sites, resulting in the points deviating negatively from a slope of unity on the right of the figure, when the actual ${}^2\alpha_{\text{chitin-environmental water}}$ rises above about 1.04. In effect, the data in Figure 4.21 actually describe a slight curve. Re-

interpreting the data with a polynomial regression (Figure 4.23, equation A) yields a better fit. However, application of this equation to a calculated versus actual ${}^2\alpha_{\text{chitin-environmental water}}$ plot still does not yield a one-to-one relationship (Figure 4.24), although it does improve the fit slightly. Experimentation with values yields equation B in Figure 4.23, which provides a reasonable fit for that data and provides a close to one-to-one relationship in a calculated versus actual ${}^2\alpha_{\text{chitin-environmental water}}$ plot (Figure 4.25). The good visual fit of a $y=x$ line in Figure 4.25 further validates this relationship.



$$\text{A: } y = (2.83 \times 10^{-5})x^2 - (2.86 \times 10^{-3}) + 1.0019 \quad r^2 = 0.583$$

$$\text{B: } y = (5.7 \times 10^{-5})x^2 - (4.5 \times 10^{-3}) + 1.008$$

Figure 4.23: Polynomial regression of the data in Figure 4.21. Line A is a mathematical regression while line B is interpretive, formulated to optimize a one-to-one calculated versus actual ${}^2\alpha_{\text{chitin-environmental water}}$ relationship (Figure 4.25) while maintaining a good fit for the above data.

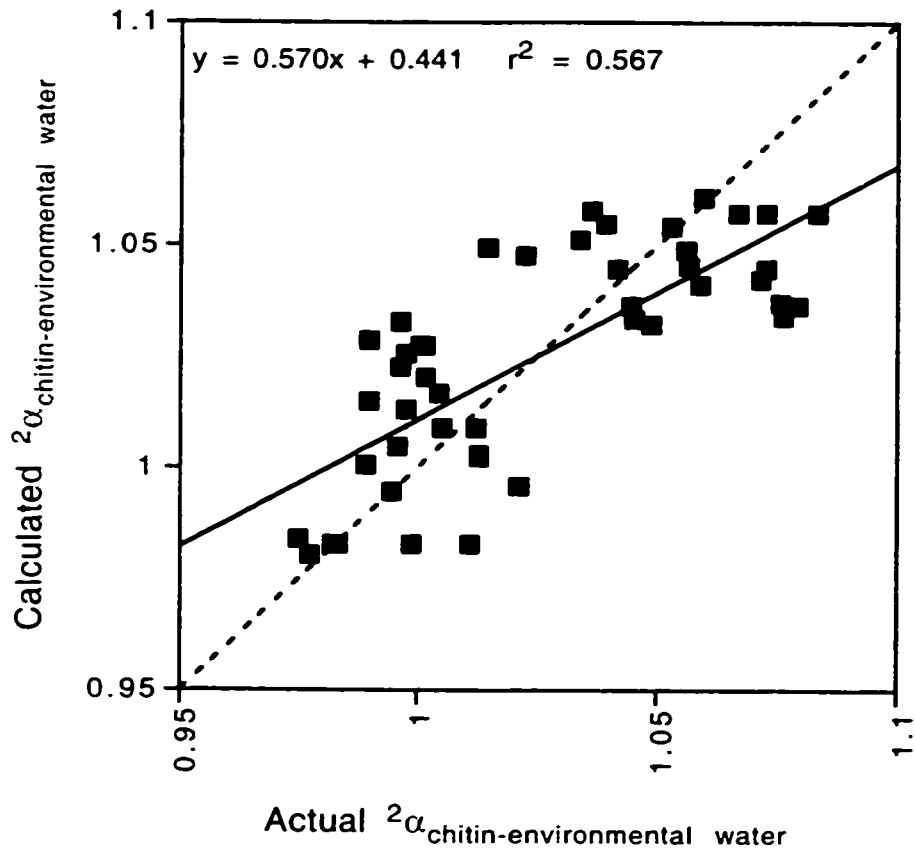


Figure 4.24: Calculated versus actual ${}^2\alpha_{\text{chitin-environmental water}}$ plot using Figure 4.23, equation A for calculated values. Dashed line is $y = x$. Once again, deviation from a one-to-one relationship shows that ${}^2\alpha_{\text{chitin-environmental water}}$ calculations do not accurately model natural processes.

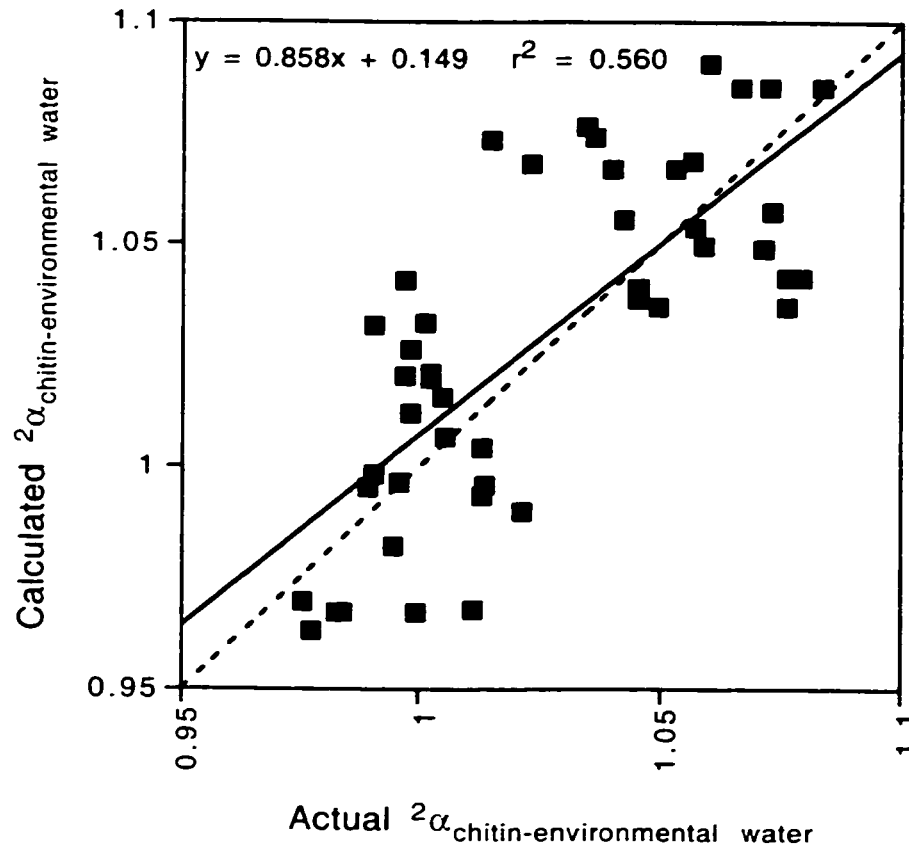


Figure 4.25: Calculated versus actual $2\alpha_{\text{chitin-environmental water}}$ plot using Figure 4.23, equation B for calculated values. Dashed line is $y = x$. This relationship approaches $y = x$, showing that the equation B $2\alpha_{\text{chitin-environmental water}}$ formula is a more accurate model.

Much of the scatter in Figure 4.25 can probably be attributed to the uneven effects of the simplifying assumptions discussed earlier and the empirical procedure used for establishing the temperature-dependent relationship. Some variability also likely results from the incomplete knowledge of insect biology and the variety of beetle families represented, particularly in the Miller *et al.* (1988) data. Despite this, it is clear that this model, with a temperature-dependent function applied,

provides a reasonable description of the hydrogen-isotopic fractionations between water and chitin.

Normalizing $\delta^2\text{H}_{\text{chitin}}$ for a 0°C annual temperature and plotting the result against $\delta^2\text{H}_{\text{environmental water}}$ (Figure 4.26) results in a linear relationship with a slope close to one. Elimination of the temperature-dependency of $\delta^2\text{H}_{\text{chitin}}$ therefore results in a direct relationship with $\delta^2\text{H}_{\text{environmental water}}$ as compared to a slope of 0.468 exhibited in Figure 4.18, where $\delta^2\text{H}_{\text{chitin}}$ was not normalized. Furthermore, the linear regression in Figure 4.26 is a closer fit than that in Figure 4.18 ($r^2 = 0.72$), indicating that the temperature-dependency of $^2\alpha_n$ accounts for some of the scatter in those data. The intercept in Figure 4.26 is inversely dependent upon the normalization temperature, dropping to zero at approximately 11°C. Normalizing $\delta^2\text{H}_{\text{chitin}}$ for a 0°C annual temperature and 100% humidity (Figure 4.27) removes some of the scatter in the data and reduces the intercept (because evaporative enrichment would be nil at 100% humidity). However, the fact that the linear fit is not improved significantly suggests that the remaining scatter is due to the factors discussed above.

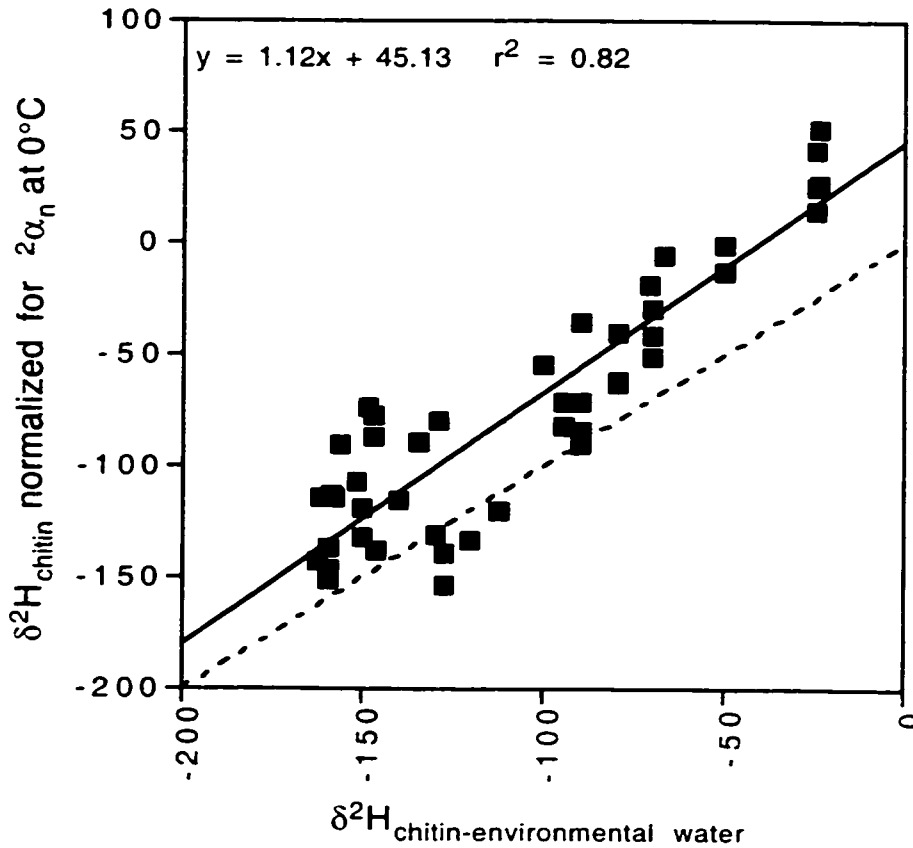


Figure 4.26: $\delta^2\text{H}_{\text{chitin}}$, normalized for $^2\alpha_n$ at 0°C annual temperature, versus $\delta^2\text{H}_{\text{environmental water}}$. Dashed line is $y = x$. Compensation for the temperature-related effect on $^2\alpha_n$ improves the linear fit of this relationship and brings the slope much closer to one.

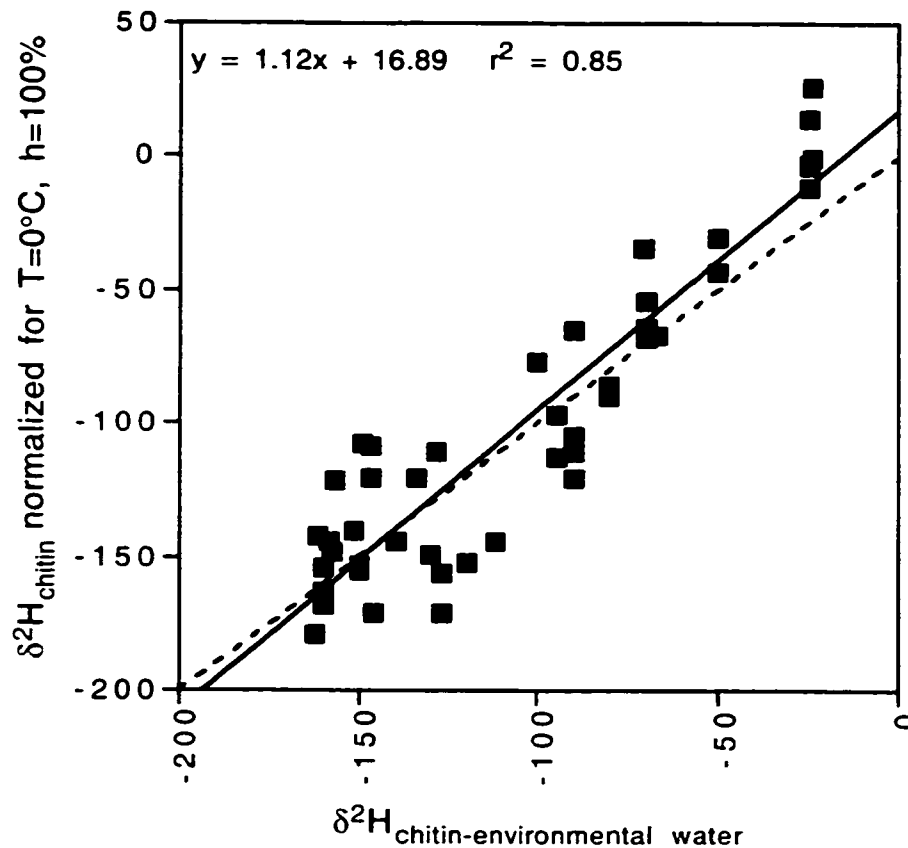


Figure 4.27: $\delta^2\text{H}_{\text{chitin}}$, normalized for $^2\alpha_n$ at 0°C annual temperature and 100% humidity, versus $\delta^2\text{H}_{\text{environmental water}}$. Dashed line is $y = x$. Further compensation for humidity-related effects brings the regression closer to $y = x$, but results in no improvement to the linear fit.

Plotting $\Delta^2\text{H}_{\text{chitin-environmental water}}$ with $\delta^2\text{H}_{\text{chitin}}$ normalized for $^2\alpha_n$ at 0°C annual temperature, against relative humidity (Figure 4.28) shows that there is a slight relationship between the enrichment of chitin over environmental water and humidity that was obscured by the influence of temperature on $^2\alpha_n$. While it is clear from Figure 4.28 that $\delta^2\text{H}_{\text{chitin}}$ becomes more enriched as humidity is reduced, it is obvious that even after normalization for temperature the relationship is poor. This may result from the simplifying assumptions used in formulating the chitin

model and the wide variety of life habits represented by the various taxa.

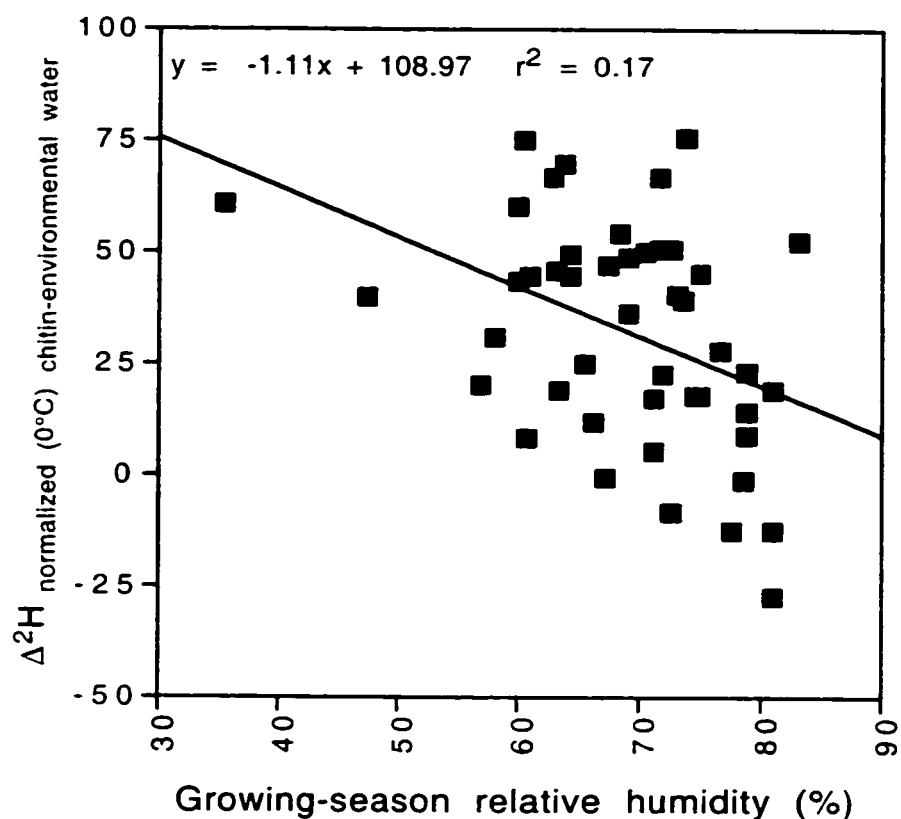


Figure 4.28: $\Delta^2\text{H}_{\text{chitin-environmental water}}$ versus RH, with $\delta^2\text{H}_{\text{chitin}}$ normalized for $^2\alpha_n$ at 0°C annual temperature. Compensation for the temperature-related effect on $^2\alpha_n$ produces a slight relationship between these factors, although there are clearly other influences producing significant scatter.

A moderate correlation exists between $\delta^2\text{H}_{\text{chitin}}$ and annual temperature (Figure 4.29). This is likely inherited from the relationship between $\delta^2\text{H}_{\text{environmental water}}$ and annual temperature since $\delta^2\text{H}$ of precipitation is known to vary directly with temperature (Dansgaard, 1964). A linear regression of $\delta^2\text{H}_{\text{environmental water}}$ versus annual temperature for the data in this study and Miller *et al.* (1988) produces the relationship

$\delta^2\text{H}_{\text{environmental water}} = 4.2 \text{ T}^\circ\text{C} - 119.7$ ($r^2 = 0.76$), which is similar to the $\delta^2\text{H}_{\text{environmental water}} = 5.6 \text{ T}^\circ\text{C} - 120$ ($r^2 = 0.90$) relationship for 11 North American IAEA stations in Yapp and Epstein (1982a). These authors also found a linear relationship of $\delta^2\text{H}_{\text{cellulose}} = 5.8 \text{ T}^\circ\text{C} - 134$ ($r^2 = 0.88$). Figure 4.29 exhibits a lower slope than these relationships. However, normalizing the $^2\alpha_n$ of $\delta^2\text{H}_{\text{chitin}}$ for an annual temperature of 0°C (Figure 4.30), which removes the apparent temperature-dependent fractionation, significantly improves the linear correlation and results in a relationship similar to those above. Further normalization to 100% humidity (Figure 4.31), to compensate for the effects of evaporative enrichment, makes the $\delta^2\text{H}_{\text{chitin}}$ and temperature relationship almost identical to that observed between $\delta^2\text{H}_{\text{environmental water}}$ and temperature in Yapp and Epstein (1982a). This indicates that the $\delta^2\text{H}_{\text{environmental water}}$ and temperature correlation is passed on to chitin through the insects' food plants.

Correction for humidity unexpectedly degrades the linear fit, an effect which appears to result from sites which deviate significantly from the $\delta^2\text{H}_{\text{environmental water}}$ - temperature relationship noted above. The most obvious example is site 31 (circled in Figures 4.30 and 4.31). It is a dry site with a relatively low $\delta^2\text{H}_{\text{environmental water}}$ for its high annual temperature. When the humidity-related enrichment is removed, the $\delta^2\text{H}_{\text{chitin}}$ value falls far below the regression line. Apparently, evaporative enrichment results in an improved correlation with temperature, under some conditions, while the noise associated with the temperature dependence of $^2\alpha_n$ degrades this relationship. It is logical that the linear fit of the $\delta^2\text{H}_{\text{chitin}}$ - temperature relationship should be reduced by mathematical manipulation which brings it closer to the $\delta^2\text{H}_{\text{environmental water}}$ - temperature relationship, since the latter has an even poorer linear correlation.

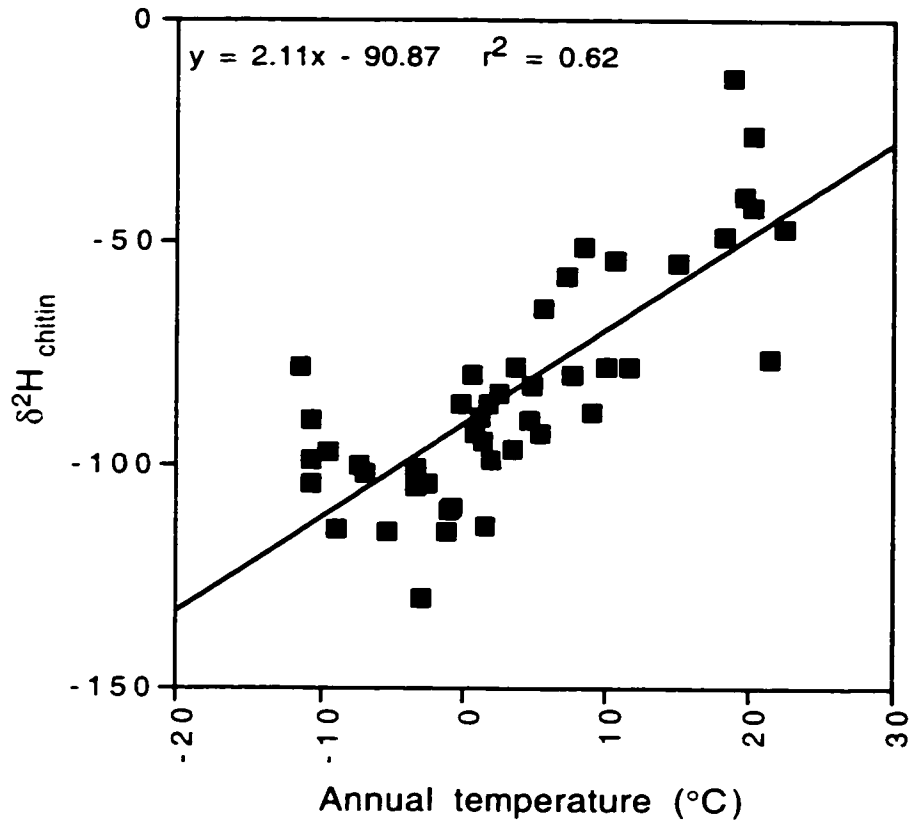


Figure 4.29: $\delta^{2}\text{H}_{\text{chitin}}$ versus annual temperature, demonstrating the linear correlation between these values.

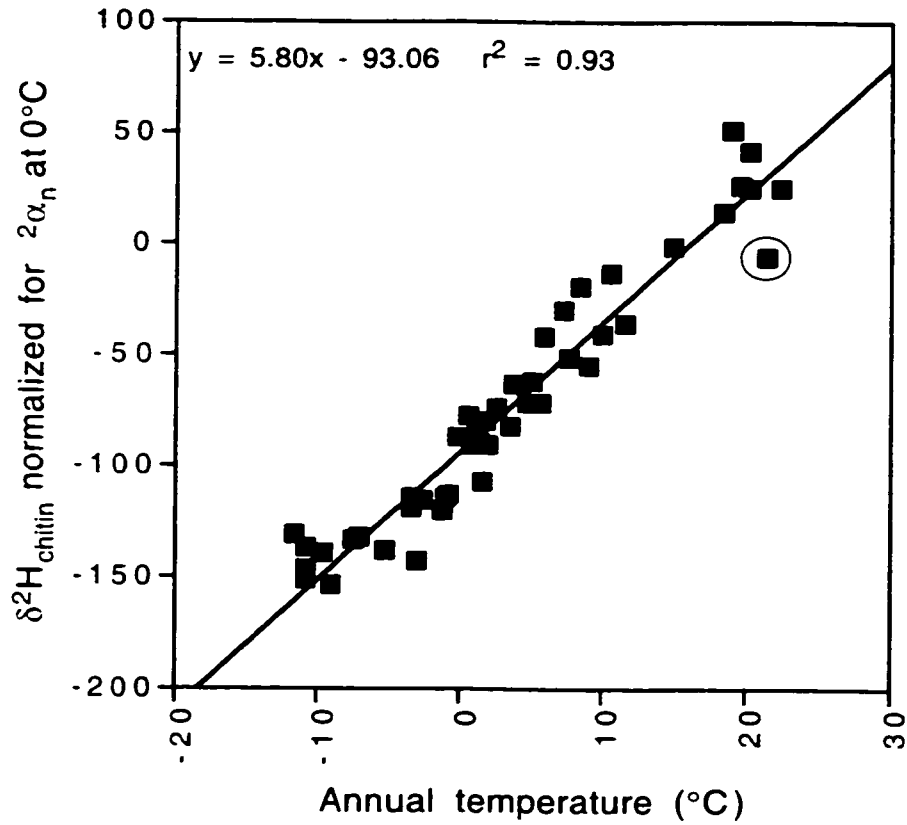


Figure 4.30: $\delta^2\text{H}_{\text{chitin}}$, normalized for $^2\alpha_n$ at 0°C annual temperature, versus annual temperature, showing that compensation for the temperature-related effect on $^2\alpha_n$ significantly improves the linear correlation. Site 31 is circled for reasons explained in the text and Figure 4.31.

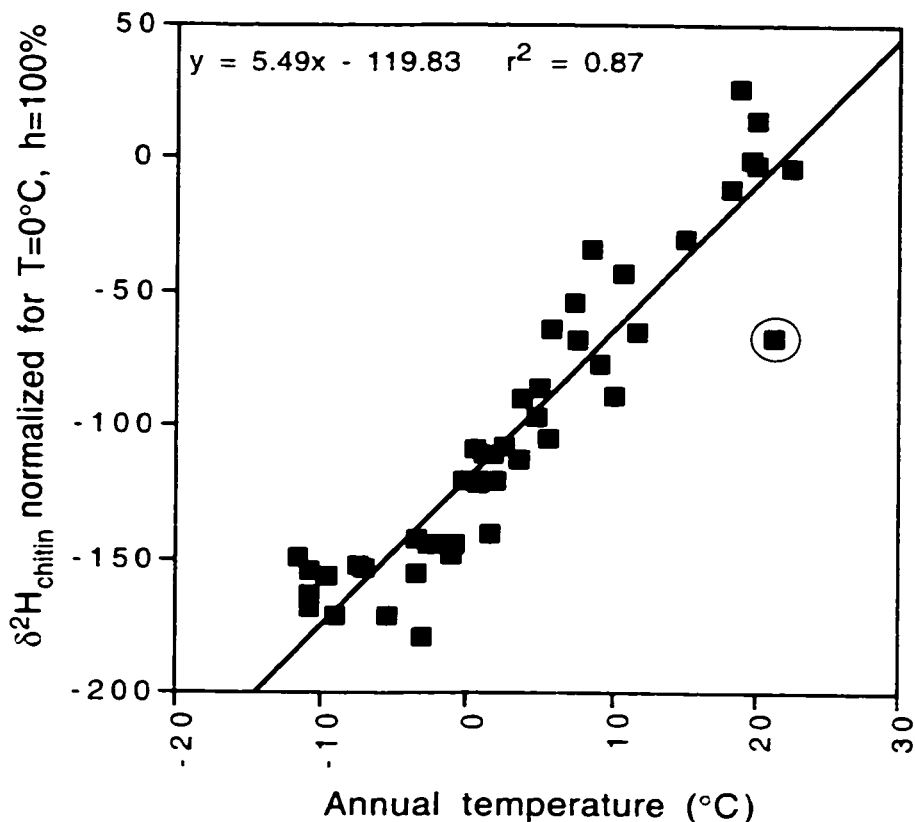


Figure 4.31: $\delta^2\text{H}_{\text{chitin}}$, normalized for $^2\alpha_n$ at 0°C annual temperature and 100% humidity, versus annual temperature. Normalization of temperature and humidity degrades the linear fit of this relationship, apparently because compensation for variations in humidity-related evaporative enrichment moves some sites, notably site 31 (circled), off the regression line.

There is a poor relationship between $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ (Figure 4.32), however, normalizing $\delta^2\text{H}_{\text{chitin}}$ for an annual temperature of 0°C (Figure 4.33) improves the linear fit significantly and brings the slope closer to one, which would be expected if temperature and humidity affected $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ equally. Calculation of an average $^2\alpha_{\text{chitin-cellulose}}$ yields 1.063 ± 0.019 . Combining this with $^2\alpha_{\text{cellulose-water}}$ of 0.953 from Edwards and Fritz (1986) results in $^2\alpha_{\text{chitin-water}}$ of 1.013, which is

similar to the ${}^2\alpha_n$ of 1.008 when the temperature dependency of $\delta^2\text{H}_{\text{chitin}}$ is removed by normalization to 0°C. This serves to validate the model for chitin derived above, however, the scatter in Figure 4.33 shows there are unresolved effects inherent in the $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ relationship.

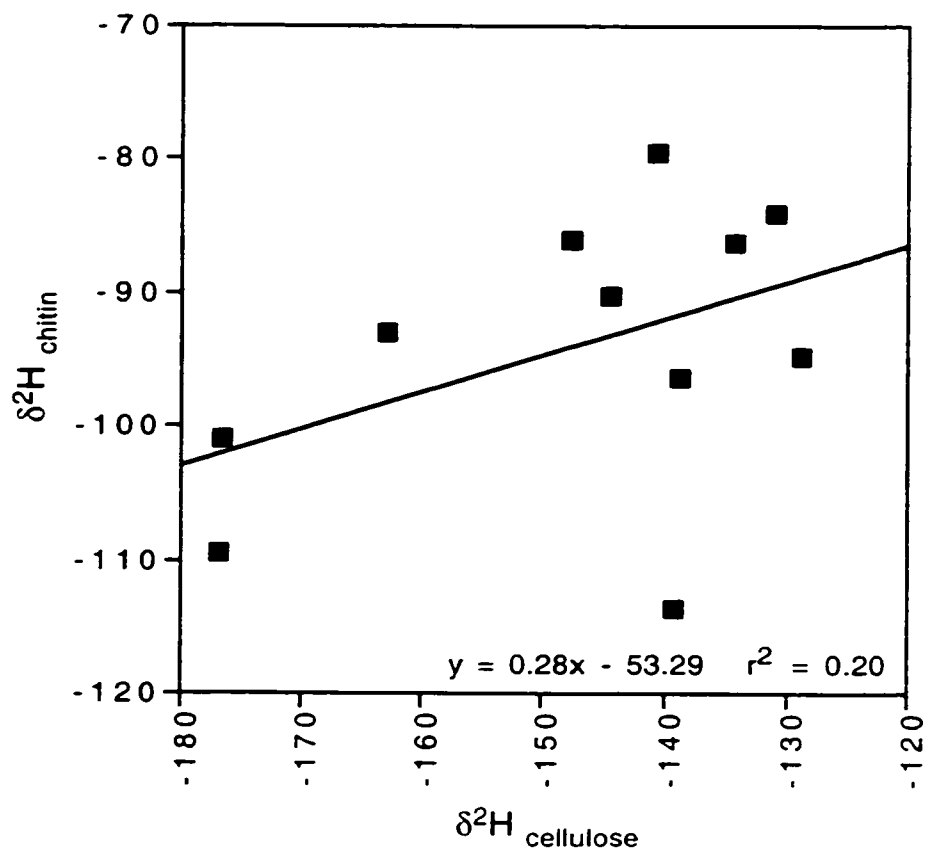


Figure 4.32: $\delta^2\text{H}_{\text{chitin}}$ versus $\delta^2\text{H}_{\text{cellulose}}$, demonstrating a poor correlation between these values.

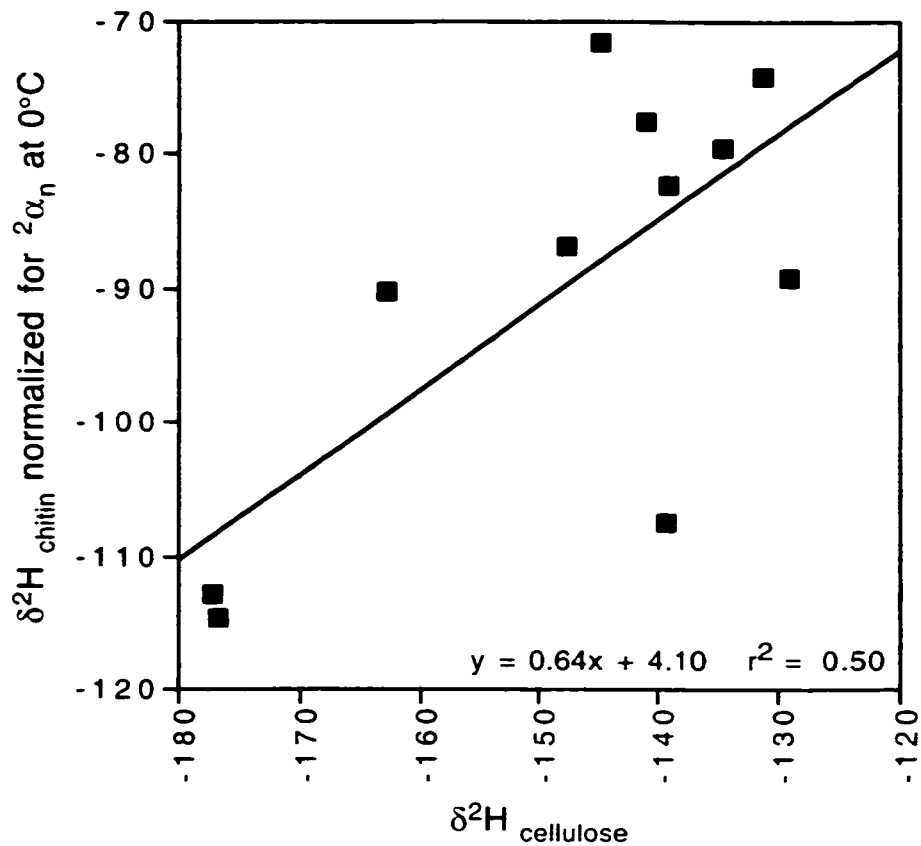


Figure 4.33: $\delta^2\text{H}_{\text{chitin}}$, normalized for $^2\alpha_n$ at 0°C annual temperature, versus $\delta^2\text{H}_{\text{cellulose}}$. Normalization removes some of the scatter in this relationship and brings the slope closer to one, however, some unresolved effects clearly remain.

4.4: Combining chitin $\delta^{18}\text{O}$ and $\delta^2\text{H}$:

Plotting $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ for chitin and cellulose (Figure 4.34) provides an illustration of the fractionating processes affecting the two isotopic species as they undergo the transformation from components of environmental water to components of chitin and cellulose. In Figure 4.34 the filled symbols are raw chitin and cellulose isotopic values, while the open symbols are those values with the net biological fractionations ($^{18}\alpha_n$ and $^2\alpha_n$) calculated out, resulting in figures for the leaf water from which cellulose and chitin are synthesized.

This process is illustrated in Figure 4.35 for sites 4 and 12. Note that the leaf water is evaporatively enriched, leading to offsets from the global meteoric water line (GMWL) along evaporation lines with slopes dependent on local conditions (Gibson, 1991) which are distinct for each site. The average slope of the evaporation lines for all sites is 2.96 ± 2.14 for chitin and 2.50 ± 1.63 for cellulose. These leaf water values cluster along a line roughly paralleling the GMWL, but offset to the right by the effects of evaporative enrichment. If the slope of the evaporation line applicable to a site is known, then a predictive capability is imparted to this process, whereby leaf water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ calculated from chitin or cellulose can be extrapolated down the local evaporation line to its intersection with the GMWL, at the point of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for the site's environmental water.

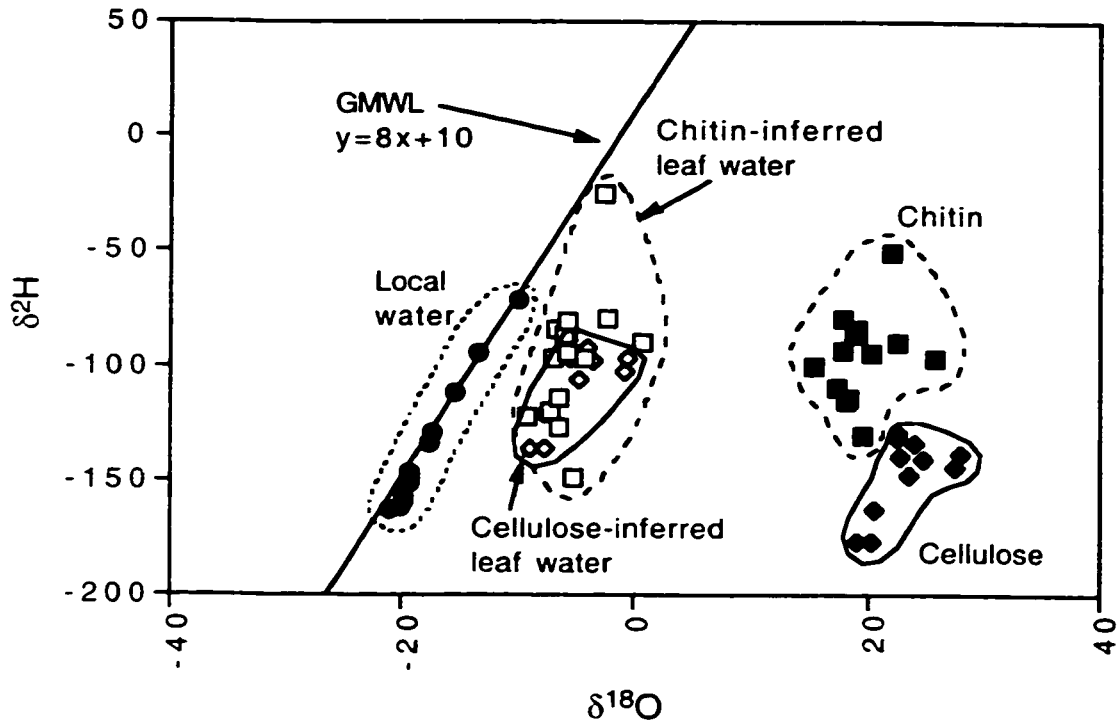
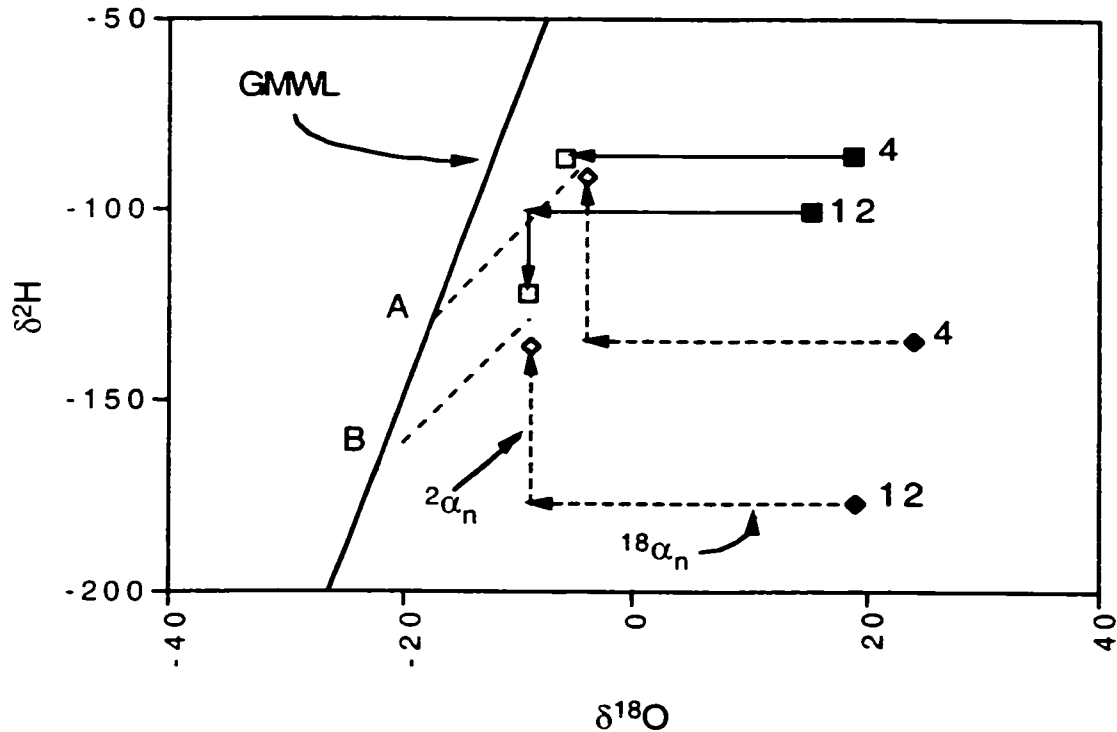


Figure 4.34: $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ for chitin and cellulose from sites 1 to 14. The global meteoric water line (GMWL) is constructed such that $\delta^2\text{H} = 8\delta^{18}\text{O} + 10$. Filled symbols are raw chitin and cellulose isotopic values, open symbols are leaf-water figures, calculated from the chitin and cellulose models, based on $(1000 + \delta_{\text{sample}})/(1000 + \delta_{\text{leaf water}}) = \alpha_n$.



Evaporation line A: $y = 3.13x - 75.48$
 Evaporation line B: $y = 2.98x - 102.43$
 GMWL: $y = 8x + 10$

Figure 4.35: Illustration of the process for deriving evaporated leaf water values from $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for chitin and cellulose by calculating out biological fractionations for sites 4 and 12. The evaporation lines are plotted from the co-ordinates of the environmental waters for each site to the average leaf water value calculated from chitin and cellulose. Squares are chitin and diamonds are cellulose.

The hydrogen discussion demonstrated that annual temperature had an effect on the apparent isotopic separation between the $\delta^2\text{H}$ chitin and environmental water, probably due to preferential uptake of summer precipitation in cold climates. While no such effect was evident in the

$\Delta^{18}\text{O}_{\text{chitin-environmental water}}$, it is possible that this effect was masked by variations in humidity-related evaporative enrichment. To examine this possibility $\delta^{18}\text{O}_{\text{chitin}}$ was normalized for 100% RH and the isotopic separation between these values and $\delta^{18}\text{O}_{\text{environmental water}}$ was plotted against annual temperature (Figure 4.36). From this it is clear that there is no apparent relationship between $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ and temperature.

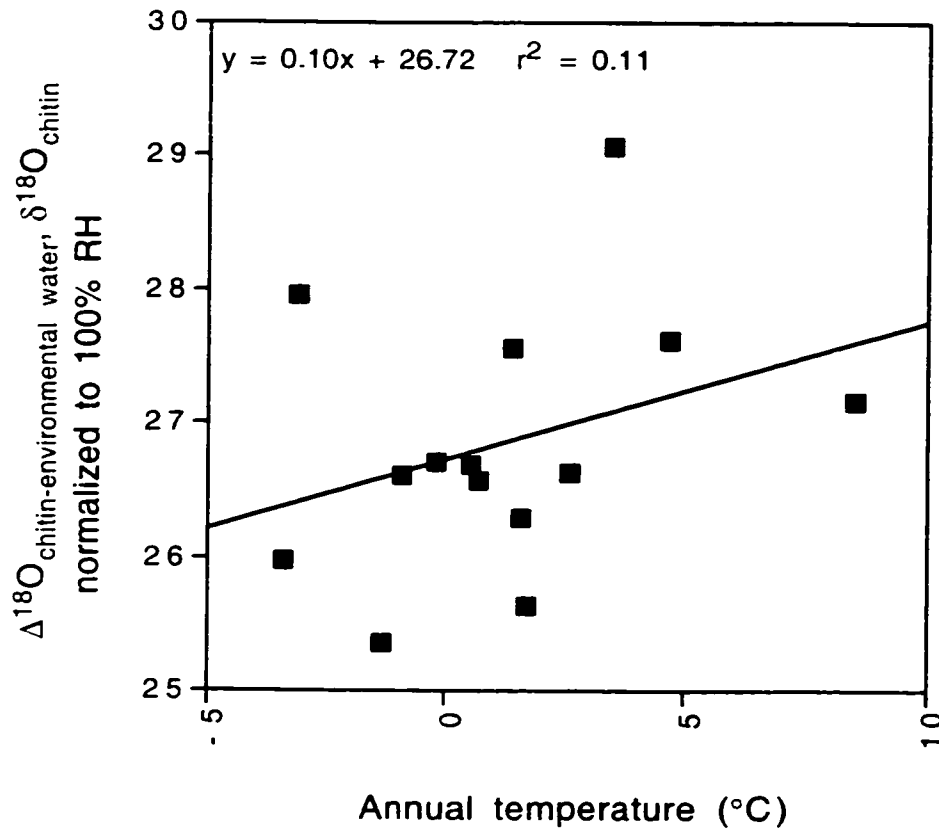


Figure 4.36: $\Delta^{18}\text{O}_{\text{chitin-environmental water}}$ with $\delta^{18}\text{O}_{\text{chitin}}$ normalized for 100% RH versus annual temperature.

Although no temperature dependency is evident in the isotopic separation between $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{environmental water}}$, it is possible that this absence is a consequence of the smaller $\delta^{18}\text{O}_{\text{chitin}}$ data set. To explore this possibility a temperature-dependent $^{18}\alpha_n$, using the same polynomial

form as that for ${}^2\alpha_n$, can be estimated. If one assumes that such a temperature effect exists, the ${}^{18}\alpha_n$ of 1.0247 derived earlier can be viewed as an average of the temperature-dependent biological fractionations. Therefore:

$$1.0247 = Y(T^\circ\text{C})^2 - Z(T^\circ\text{C}) + X \quad (4.6)$$

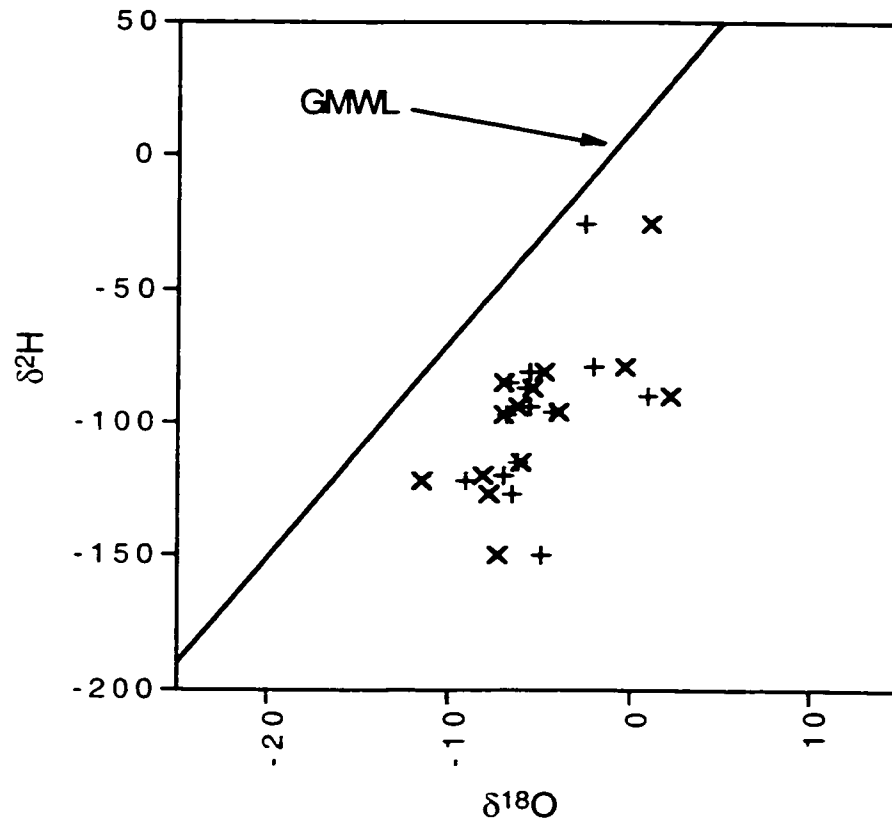
If one further assumes, based on the GMWL slope of eight, that the temperature effect on $\delta^{18}\text{O}_{\text{chitin}}$ is one eighth that on $\delta^2\text{H}_{\text{chitin}}$, then the coefficients in the ${}^2\alpha_n$ polynomial can be divided by eight to produce values for Y and Z in equation 4.6. Therefore:

$$1.0247 = (7.1 \times 10^{-6})(T^\circ\text{C})^2 - (5.6 \times 10^{-4})(T^\circ\text{C}) + X \quad (4.7)$$

Solving for X at the average annual temperature of 1.2°C for the 14 sites produces:

$${}^{18}\alpha_n = (7.1 \times 10^{-6})(T^\circ\text{C})^2 - (5.6 \times 10^{-4})(T^\circ\text{C}) + 1.025 \quad (4.8)$$

Applying equation 4.8 to the calculation of inferred leaf water values yields a slightly different relationship in a $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ graph (Figure 4.37) than that in Figure 4.34. In Figure 4.37 it is clear that the application of a temperature-dependent ${}^{18}\alpha_n$ for chitin shifts inferred leaf-water values to the right in more enriched locations and to the left in more depleted ones. The net effect is to rotate the cluster of points clockwise to bring it nearly parallel with both the GMWL and the points for cellulose-inferred leaf water values. While it is compelling to hypothesize that, if a temperature effect is apparent in $\delta^2\text{H}_{\text{chitin}}$, it should also show up in $\delta^{18}\text{O}_{\text{chitin}}$, the absence of field evidence for this dependency and the necessity of deriving a numerical description from ${}^2\alpha_n$ make its use speculative. Confirmation of this effect will have to wait until a $\delta^{18}\text{O}_{\text{chitin}}$ data set similar in scope to that for $\delta^2\text{H}_{\text{chitin}}$ is available.



- | | | | |
|---|---|---|--|
| x | Leaf water inferred from chitin with $^{18}\alpha_n$ temperature effect | + | Leaf water inferred from chitin without $^{18}\alpha_n$ temperature effect |
|---|---|---|--|

Figure 4.37: $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ for leaf water inferred from chitin, with $\delta^{18}\text{O}_{\text{leaf water}}$ calculated using fixed and temperature-dependent $^{18}\alpha_n$ factors.

Based on the model for chitin derived in this chapter, it should be possible to formulate a methodology to infer environmental water isotopic values and humidity from $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$. The following expressions relating chitin isotopic composition and environmental parameters were derived previously:

$$\frac{1000 + \delta^{18}\text{O}_{\text{chitin}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}} = {}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k - {}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)h \quad (4.9)$$

Where ${}^{18}\alpha_n = 1.0247$, ${}^{18}\alpha_e = 1.0095$ and ${}^{18}\alpha_k = 1.0260$.

$$\frac{1000 + \delta^2\text{H}_{\text{chitin}}}{1000 + \delta^2\text{H}_{\text{environmental water}}} = {}^2\alpha_n {}^2\alpha_e {}^2\alpha_k - {}^2\alpha_n ({}^2\alpha_e {}^2\alpha_k - 1)h \quad (4.10)$$

Where ${}^2\alpha_n = (5.7 \times 10^{-5})(T^\circ\text{C})^2 - (4.5 \times 10^{-3})(T^\circ\text{C}) + 1.008$,
 ${}^2\alpha_e = 1.0797$ and ${}^2\alpha_k = 1.0186$.

Using the equation for the global meteoric water line, $\delta^2\text{H}_{\text{environmental water}} = 8 \delta^{18}\text{O}_{\text{environmental water}} + 10$ (Craig, 1961), to substitute for $\delta^2\text{H}_{\text{environmental water}}$ in equation 4.10, two equations and two unknowns result. These can be solved graphically, as illustrated in Figure 4.38. The lines were generated using fixed $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ values, combined with relative humidities from 0 to 100% to produce $\delta^{18}\text{O}_{\text{environmental water}}$ figures. Since ${}^2\alpha_n = (5.7 \times 10^{-5})(T^\circ\text{C})^2 - (4.5 \times 10^{-3})(T^\circ\text{C}) + 1.008$, the lines for $\delta^2\text{H}_{\text{chitin}}$ were calculated assuming $T = 0^\circ\text{C}$. This graph could be used to infer $\delta^{18}\text{O}_{\text{environmental water}}$ and relative humidity from any pair of $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ values, provided the annual temperature at the sample site is 0°C .

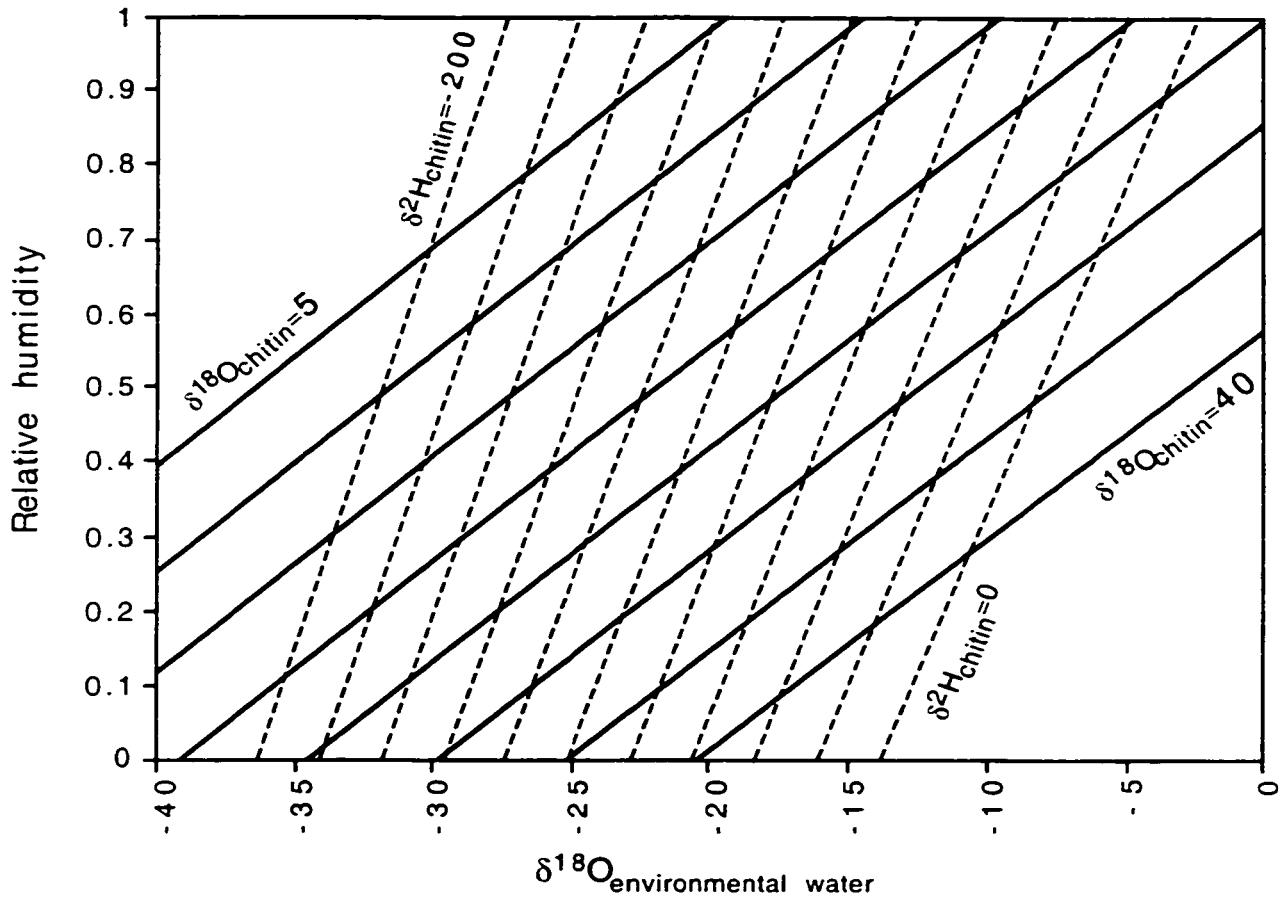


Figure 4.38: $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ model. Solid lines represent the relationship between RH and $\delta^{18}\text{O}_{\text{environmental water}}$ at fixed $\delta^{18}\text{O}_{\text{chitin}}$ values from 5‰ to 40‰. Dashed lines represent the relationship between RH and $\delta^{18}\text{O}_{\text{environmental water}}$ at fixed $\delta^2\text{H}_{\text{chitin}}$ values from -200‰ to 0‰ and $T = 0^\circ\text{C}$.

This model is sensitive to variations in input temperature: At $\delta^{18}\text{O}_{\text{chitin}} = 5$ and $\delta^2\text{H}_{\text{chitin}} = -200$, $\delta^{18}\text{O}_{\text{environmental water}}$ varies by 0.58‰/°C and humidity varies by 1.7%/°C; at $\delta^{18}\text{O}_{\text{chitin}} = 20$ and $\delta^2\text{H}_{\text{chitin}} = -100$, $\delta^{18}\text{O}_{\text{environmental water}}$ varies by 0.67‰/°C and humidity varies by 1.9%/°C; at $\delta^{18}\text{O}_{\text{chitin}} = 40$ and $\delta^2\text{H}_{\text{chitin}} = 0$, $\delta^{18}\text{O}_{\text{environmental water}}$ varies by 0.76‰/°C and

humidity varies by 2.2%/°C. Clearly, this limits its utility. However, if the temperature of a site can be estimated, using techniques such as palynology or taxonomic paleoentomology, then the same methodology can be used to plot two lines, based on measured $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ and a series of humidity values. The intersection of these two lines, on a graph similar to Figure 4.38, would produce the $\delta^{18}\text{O}_{\text{environmental water}}$ and relative humidity for those $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ values at the estimated temperature. The inferred humidity and estimated temperature could then be used in equation 4.10 to calculate $\delta^2\text{H}_{\text{environmental water}}$. This calculation would also be sensitive to input temperature, directly (in equation 4.10) and through the inferred humidity. Assuming a $\delta^2\text{H}_{\text{chitin}}$ of -58‰, inferred $\delta^2\text{H}_{\text{environmental water}}$ varies by about 3.61‰/°C. It varies by about 0.85‰/% RH, which translates into 1.61‰/°C, using the average of 1.9%/°C for the three values of inferred humidity sensitivity discussed above.

Using the chitin and cellulose models to calculate environmental water isotopic contents and humidities from the isotopic values of chitin (sites 1 to 14) and cellulose (sites 1, 2 and 4 to 12), and comparing these figures to modern data, suggests precisions for chitin inferences of about $\pm 3\text{‰}$ for $\delta^{18}\text{O}_{\text{environmental water}}$, $\pm 30\text{‰}$ for $\delta^2\text{H}_{\text{environmental water}}$ and $\pm 10\%$ for relative humidity; and for cellulose inferences of about $\pm 3\text{‰}$ for $\delta^{18}\text{O}_{\text{environmental water}}$, $\pm 30\text{‰}$ for $\delta^2\text{H}_{\text{environmental water}}$ and $\pm 8\%$ for relative humidity. These are comparable to the $\pm 2\text{‰}$ for $\delta^{18}\text{O}_{\text{environmental water}}$ and $\pm 8\%$ for relative humidity inferred from cellulose in Edwards and Fritz (1986). It is likely that the precision for both models is actually greater because modern environmental water isotopic content and humidity are not from the exact locations from which insects and wood were collected and modern observations are from different time periods than those integrated by the biological materials. In the case of $\delta^2\text{H}_{\text{environmental water}}$ inferred from chitin, the precision is probably reduced by application of the empirically defined ${}^2\alpha_n$ temperature dependency. Also, the other fractionation factors are, by necessity, generalizations which likely have differing degrees of applicability to the various sites.

**Chapter 5:
Application of chitin $\delta^{18}\text{O}$ and $\delta^2\text{H}$ to fossil material and
comparison with cellulose $\delta^{18}\text{O}$ and $\delta^2\text{H}$ and conventional
paleoentomological analysis**

In order to test the utility of isotope paleoentomological analysis the methodology detailed in foregoing sections must be applied to a fossil site. The only previous published application of chitin isotope analysis to fossil insect material was in a study of a 12,000-10,000 BP site in Lismore, Nova Scotia (Schimmelmann, *et al.*, 1993). It concluded that variations in chitin $\delta^2\text{H}$, and summer temperature derived from it, suggested climatic trends similar to those found in some late-glacial sequences in Europe, and in palynological evidence from eastern Canada. This study was, however, limited to five data points and did not look at chitin $\delta^{18}\text{O}$ or make comparisons with isotopes from cellulose or conventional paleoentomology.

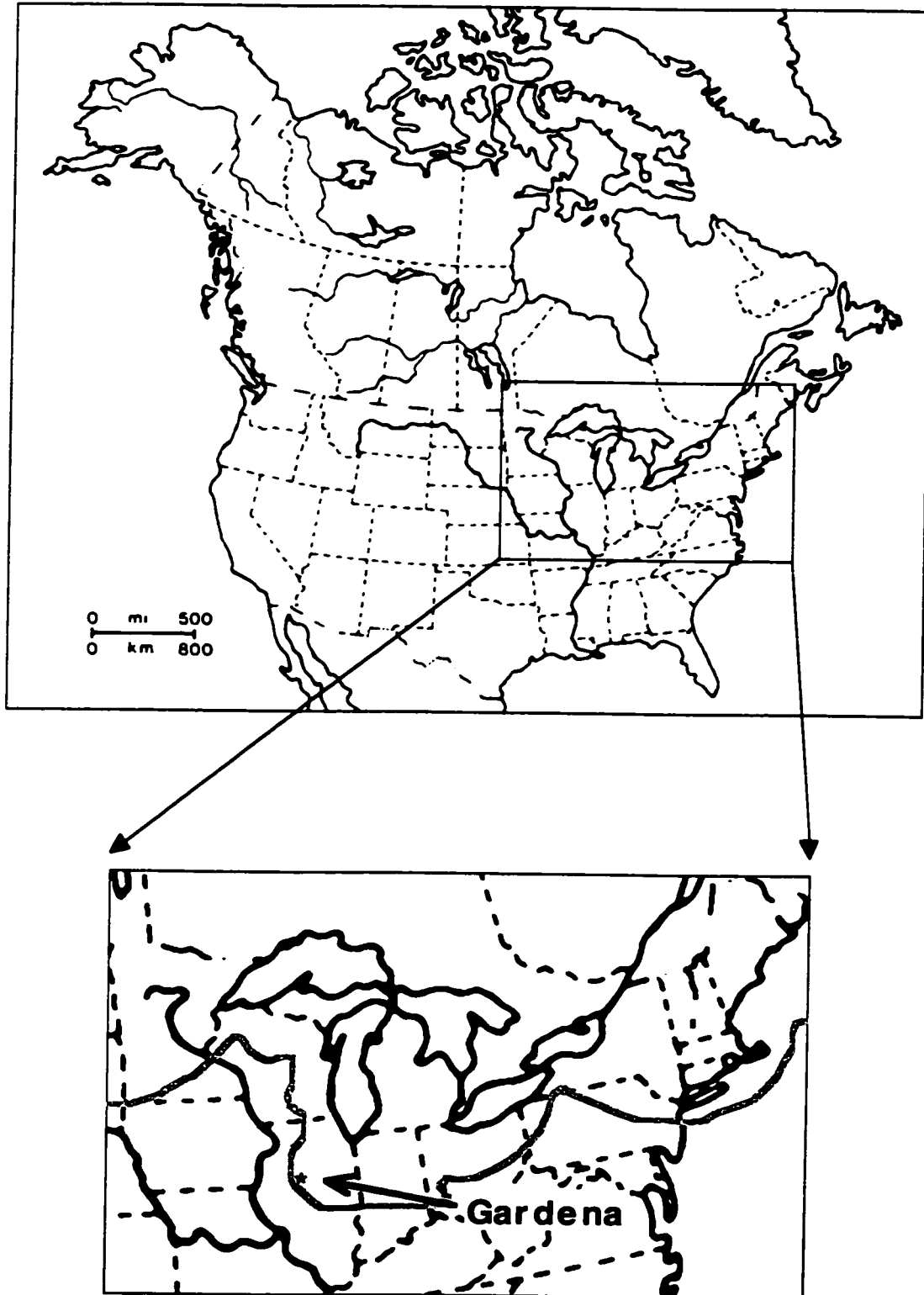


Figure 5.1: Location of Gardena site in Illinois. Gray line is approximate ice front at 18,000 BP (Morgan, 1987).

5.1: Site:

The site chosen for this study was the Gardena section, near Peoria in central Illinois (Figure 5.1). An earlier, more limited, taxonomic paleoentomological analysis was undertaken at this site (Morgan, 1987), the results of which are discussed in the concluding portion of this chapter. The section sampled consists of approximately 2.3 m of sub-till organic sediments, with radiocarbon ages ranging from 19,680 BP at the top to 25,960 BP near the base (Table 5.2) (Lineback, *et al.*, 1979) exposed in a creek bank. Underlying the sample zone is inorganic Roxana Silt, deposited during the Early Wisconsinan Altonian Substage, between 45,000 and 30,000 BP. It consists of loess blown from the outwash of glaciers which occupied northern Illinois at the time. The Roxana Silt is overlain by the organic Robein Silt, which comprises the lowest part of the sample zone. This is overlain by organic, followed by inorganic, silts of the proglacial Morton Loess, deposited, beginning about 25,000 BP, when glaciers re-entered the Ancient Mississippi drainage basin north of Peoria. Woodfordian glaciers reached the sample site at about 19,000 BP, laying down the Delavan Till Member of the Wedron Formation and ending organic deposition (Lineback, *et al.*, 1979).

A sampling datum was established at the base of the Delavan Till. Samples consisted of a bulk sample of the 5 cm-thick moss horizon, which is immediately below the till, and further samples at 10 cm intervals from 1.6 to 2.3 m below the till. The upper 1.5 m of the Morton Loess was apparently inorganic and was not sampled. Sample weights are in Table 5.1.

Horizon (metres below till)	Weight (kg)
0 (moss)	28.84
1.6-1.7	31.77
1.7-1.8	33.95
1.8-1.9	33.62
1.9-2.0	31.52
2.0-2.1	29.67
2.1-2.2	29.8
2.2-2.3	31.03

Table 5.1: Sample depths and weights.

5.2: Laboratory procedures:

Samples were collected in polyethylene bags and weighed. Since the sediment was rich in fine material, samples were soaked in a solution of Calgon to deflocculate any clay. Processing to separate insect fossils was then carried out using the procedure described in Morgan and Morgan (1980a) (Appendix 4). Plant material (principally twigs) was removed during this procedure and stored separately. Insect fossils were sorted in ethanol under a binocular microscope. Those which appeared to be identifiable were mounted on micropaleontological slides, while miscellaneous fragments were stored in ethanol-filled vials. The author performed preliminary identifications on the mounted fossils, which were then sent to taxonomic experts for final identification (Table 5.2).

Once identification was complete, the insect samples were purified to chitin and analyzed for oxygen and hydrogen isotopes using the procedures described previously. Plant material was finely ground, purified to cellulose and analyzed for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ using previously-described techniques. TIG-welded nickel tubes (Experiment 3 apparatus from Chapter 2) were used for all analyses.

5.3: Taxonomic analysis of Coleopteran fossils:

Depth below till		0m	1m	2 m						
Radiocarbon dates		A	B			C	D			
Stratigraphic units		moss		inorganic silt			organic silt			
Taxon	Dist.	Level								
		0	unsampled	1.6	1.7	1.8	1.9	2.0	2.1	2.2
Carabidae										
<i>Agonum/Pterostichus</i> sp.										
<i>Bembidion</i> spp.										
<i>Dyschirius</i> spp.										
possibly <i>Dyschirius hiemalis</i> Bousquet	1-2									
<i>Elaphropus</i> sp.										
<i>Elaphrus lapponicus</i> lapponicus Gyllenhal	1-2									
<i>Sphaeroderus nitidicollis brevoorti</i> LeConte	4									
gen. indet.										
Dysticidae										
cf. <i>Hydroporus</i>										
cf. <i>Oreodytes laevis</i> (Kirby)	2									
gen. indet.										
Hydraenidae										
<i>Hydraena</i> sp.										
Staphylinidae										
<i>Acidota quadrata</i> (Zetterstedt)	2									
<i>Aleocharinae</i> spp.										
<i>Bryophacis smetanae</i> Campbell	2-3									

Table 5.2: Continued on next page. Radiocarbon dates are: A=19,680±460 (moss, ISGS-532), B=25,680±1,000 (wood, ISGS-530), C=25,370±310 (wood, ISGS-531) and D=25,960±280 (wood, ISGS-529). Note that the depth is not to scale.

Taxon	Dist.	Level								
		0	unsampled	1.6	1.7	1.8	1.9	2.0	2.1	2.2
<i>Eucnecosum brachypterum</i> (Gravenhorst)	1									
<i>Eucnecosum brunnescens</i> (J. Sahlberg)	2									
<i>Gymnusa campbelli/pseudovariegata</i>	2									
<i>Lathrobioma</i> sp.										
<i>Mycetoporus/Bryophacis</i> sp.										
<i>Olophrum</i> spp.										
<i>Olophrum latum</i> Mäklin	1									
<i>Olophrum rotundicolle</i> (C. R. Sahlberg)	2									
<i>Omaliinae</i> sp.										
<i>Philonthus</i> spp.										
<i>Stenus hyperboreus</i> J. Sahlberg	2-3									
<i>Stenus</i> spp. gen. indet.										
Pselaphidae										
possibly <i>Tyrus humeralis</i> (Aubé)	2-3									
<i>Tyrus humeralis</i> (Aubé)	2-3									
Hydrophilidae										
<i>Helophorus</i> sp.										
<i>Hydrochus</i> sp. gen. indet.										
Scarabidae										
gen. indet.										
Byrrhidae										
possibly <i>Cytilus</i> sp.										

Table 5.2: Continued on next page.

Taxon	Dist.	Level								
		0	unsampled	1.6	1.7	1.8	1.9	2.0	2.1	2.2
Elateridae										
gen. indet.										
Cucujidae										
gen. indet.										
Ciidae										
gen. indet.										
Apionidae										
cf. <i>Apion</i> sp.										
Curculionidae										
gen. indet.										
Scolytidae										
<i>Carphoborus andersoni</i>	1									
Swaine										
possibly <i>Cryphalus ruficollis</i>	3									
Hopkins										
<i>Ips latidens</i> (LeConte)	2-3									
possibly <i>Ips pini</i> (Say)	3									
<i>Pityophthorus</i> spp.										
<i>Polygraphus rufipennis</i>	3									
(Kirby)										
<i>Scolytus</i> sp.										
<i>Scolytus picea</i> (Swaine)	3									
gen. indet.										
Coleoptera										
fam. indet.										
Total number of individuals	261									

Table 5.2: Coleopteran taxa recovered from the Gardena site. Distribution categories are explained in the text. Species intermediate between categories were assigned hyphenated distributions.

The modern geographic distributions of the Gardena beetles were divided into a series of categories, referred to in Table 5.2 and described as follows:

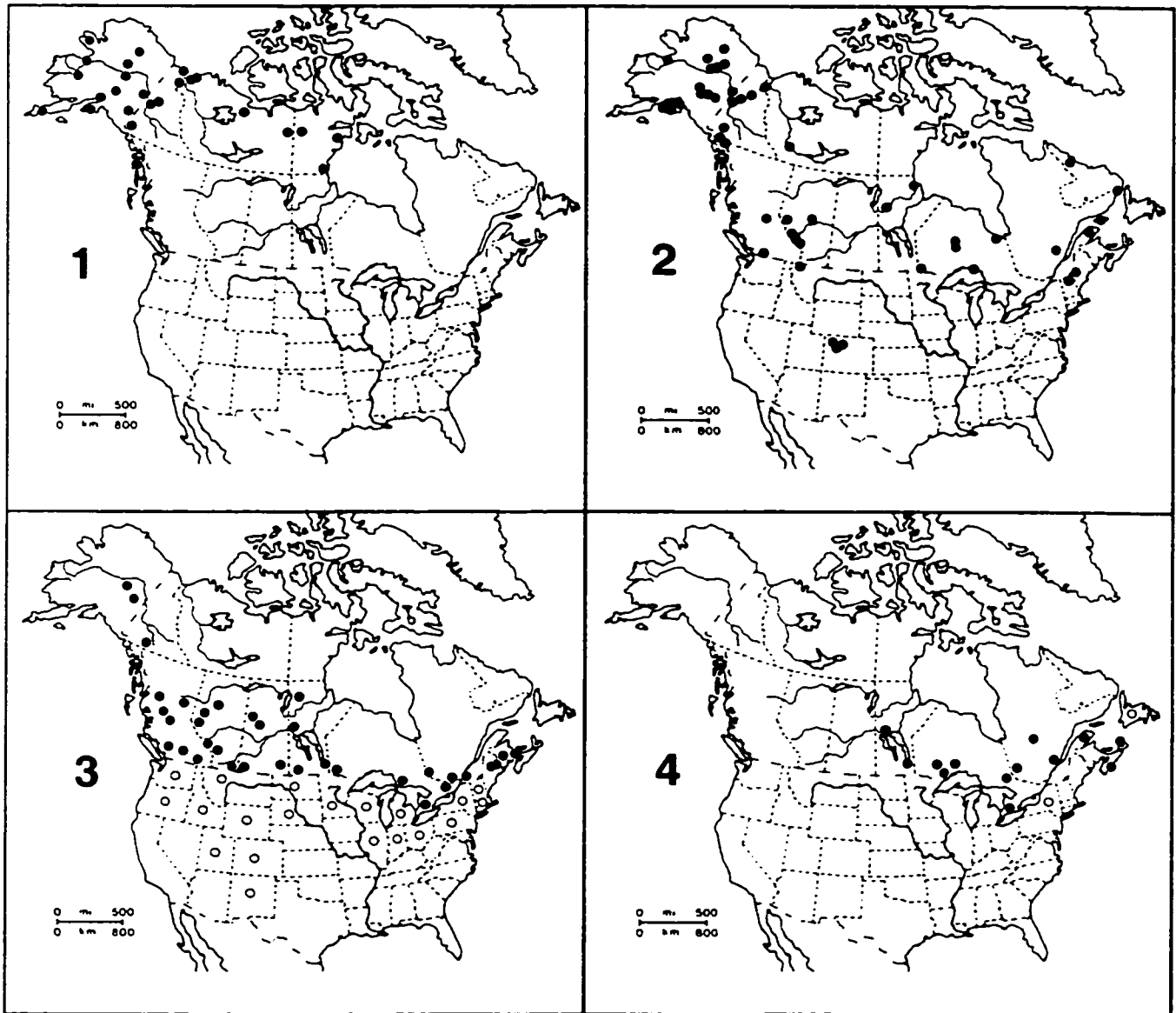


Figure 5.2: The four distribution categories used in Table 5.2: 1. *Olophrum latum*; 2. *Acidota quadrata*; 3. *Scolytus picea*; and 4. *Sphaeroderus nitidicollis brevoorti*. Solid points are exact collecting localities, open points are state or provincial records.

Distribution 1: Beetles whose modern distribution is restricted to northern boreal, tundra and high-altitude areas, with mean annual temperatures ≤ -5 °C and mean July temperatures ≤ 15 °C. *Olophrum latum* (Figure 5.2) (Campbell, 1983) is an example of such a distribution.

Distribution 2: Species with modern distributions extending from northern boreal, treeline or tundra habitats to the southern limit of the boreal forest or the northern portion of the prairies. These insects are found in areas with mean annual temperatures ≤ 3 °C and mean July temperatures ≤ 17 °C and do not occur in southernmost Ontario today. *Acidota quadrata* (Figure 5.2) (Campbell, 1982) has this type of distribution.

Distribution 3: Species which occur from the central boreal forest south to southernmost Canada and the northern United States, in areas with mean annual temperatures ≥ -5 °C and mean July temperatures ≥ 15 °C. An example of this distribution *Scolytus picea* (Figure 5.2) (Bright, 1976).

Distribution 4: Individuals with modern distributions extending from the southern boreal forest or northern prairies into southernmost Canada and the northern United States. These areas have mean annual temperatures ≥ 0 °C and mean July temperatures ≥ 17 °C. *Sphaeroderus nitidicollis brevoorti* (Figure 5.2) (Lindroth, 1961) exhibits this distribution.

Species intermediate between categories were assigned hyphenated distributions. Temperature requirements of the various distribution categories were determined by comparing the geographic ranges of the insects with the isothermal contour maps in the Climatic Atlas-Canada (Anonymous, 1984).

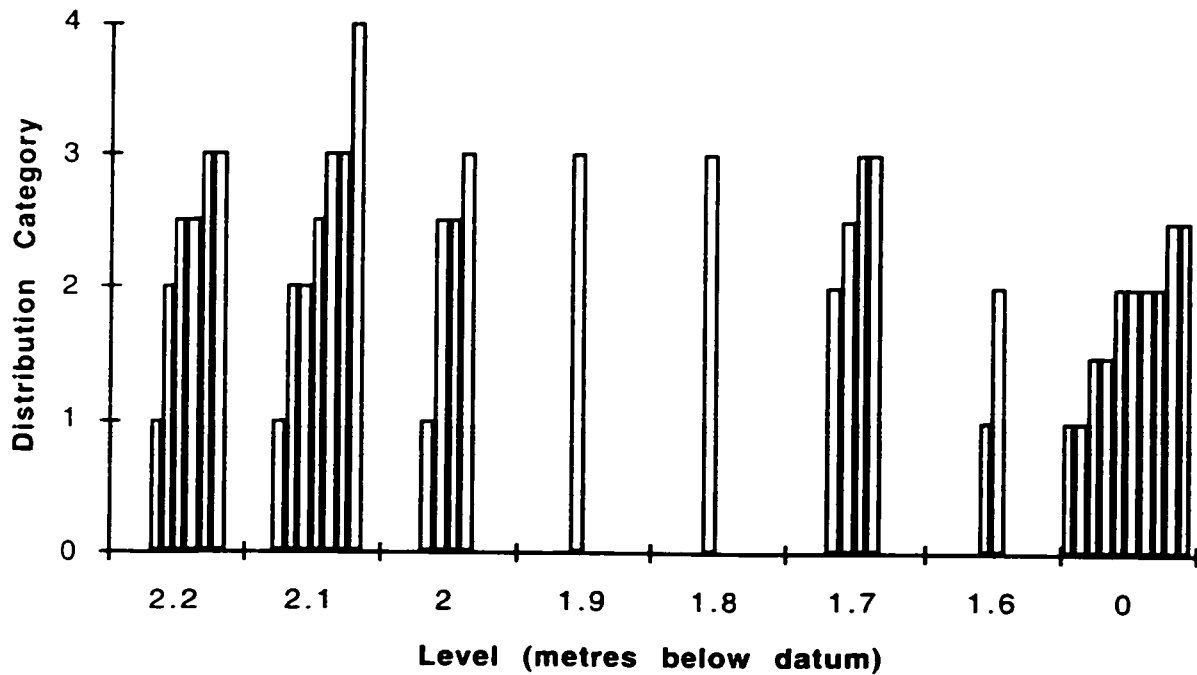


Figure 5.3: Distribution-category content for the various levels of the Gardena site. Each bar represents a specifically-identified individual from a particular level, while the length of the bar indicates which distribution category that individual is from. Multiple bars of the same length in one level indicate that more than one individual of that distribution was found there. Bars ending midway between two values represent hyphenated distributions (see Table 5.2 caption).

Figure 5.3 shows that all levels have species that can live in a wide-variety of habitats. However, it is clear that there is a trend toward colder-climate insects as one progresses up the section. Of note is the fact that levels 2.2m to 1.6m contain a variety of Scolytidae (bark beetles) which feed on trees, particularly conifers (Bright, 1976). This,

along with the abundance of wood in these levels, suggests the presence of a boreal forest in the area during that period.

The 2.2m (basal) level contains a relatively northern assemblage, with a Distribution 1 individual limiting the maximum mean annual and July temperatures to approximately -5°C and 15°C respectively, although, as discussed below, the fact that this individual is *Carphoborus andersoni*, may mean conditions were somewhat warmer. Bark beetles from this horizon, including *Scolytus picea* and *Ips latidens*, are hosted by a variety of conifers, such as *Pinus*, *Picea*, *Tsuga canadensis* and *Pseudotsuga manziesii* (Bright, 1976). *Olophrum rotundicolle*, the only non-scolytid specifically identified from this level, inhabits vegetation or plant debris along the margins of water (Campbell, 1983).

By 2.1m the area was clearly warmer, as indicated by the presence of a Distribution 4 beetle. However, the presence of a Distribution 1 form indicates that the climatic amelioration may have been moderate. Estimating a temperature from this assemblage is problematic, since there is no overlap in the modern ranges of the Distribution 1 *C. andersoni* and the Distribution 4 *Sphaeroderus nitidicollis brevoorti* found at this level. The current and fossil ranges of *C. andersoni* provide a clue to the puzzle: Currently, this insect is restricted to northwestern areas (Figure 5.4), however, it is found in fossil sites farther to the southeast, including some in the vicinity of the Great Lakes (Morgan and Morgan, 1980b; Morgan and Motz, 1995). Indeed, a fossil specimen, from Brampton in southern Ontario, dating from 4,800 BP when conditions were likely as warm as the present (Motz, 1990; Morgan and Motz, 1995), indicates that this insect can survive in temperate conditions. Likely, the population that included the individuals in this, and other, fossil sites was part of a group that was cut off from a population in a northwestern refugium by Wisconsinan ice and this insect still survives in temperate areas today but is rare and difficult to find, allowing it to escape detection in these localities (Motz, 1990; Morgan and Motz, 1995). Thus, the Gardena specimens may not signify a temperature regime like the current far north, but rather a geographic anomaly. If one accepts these conjectures, then the mean temperature range of this level would

have been restricted by the co-occurrence of Distribution 2 and 4 forms to between 0 °C and 3°C annually, and 17°C in July.

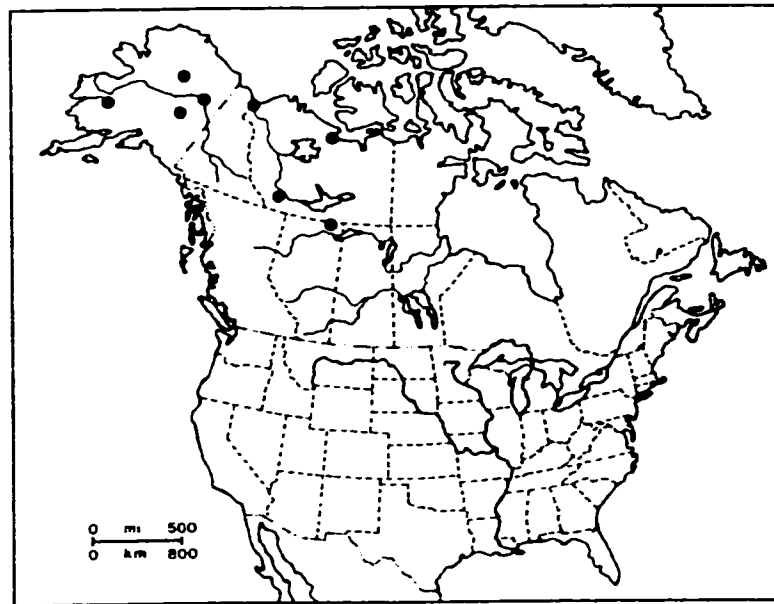


Figure 5.4: Modern distribution of *C. andersoni* (Bright, 1976; Morgan and Motz, 1995).

The Scolytidae from the 2.1m level suggest a variety of conifers was present with little change, if any from the previous horizon. Staphylinidae present, *Gymnusa campbelli/pseudovariegata* (Klimaszewski, 1979) and *Olophrum rotundicolle* (Campbell, 1983) suggest vegetation, moss or debris on the shore of stagnant or slow-moving water.

Moving up the section, the climate had cooled by 2.0m, as shown by the lack of Distribution 4 forms. The Distribution 1 individual suggests mean temperatures of ≤ 5 °C annually and ≤ 15 °C in July, although, as discussed above, since this species is *C. andersoni* temperatures may have been higher.

The insects indicate little change between 1.9m and 1.7m. The lack of cold-climate, Distribution 1 forms is suggestive of somewhat warmer mean temperatures: higher than -5 °C annually and 15 °C in July. However, these assemblages are very sparse, so any interpretation must be exercised cautiously.

By 1.6m the climate had cooled, leaving conditions suitable for only Distribution 1 and 2 species. Although the Distribution 1 type is again *C. andersoni*, the lack of any warm or even temperate forms in this horizon may mean conditions were cooling to the ≤ 5 °C mean annual and ≤ 15 °C mean July temperatures typical of this species today.

The assemblage from the 0m (moss) level is markedly different from the rest of the section and exhibits a notable environmental shift. It is dominated by colder Distribution 1, 2 and intermediate types. Furthermore, there is a variety of Distribution 1 and 1-2 species, giving them greater weight as temperature indicators. These factors show cooling by this point to ≤ 5 °C mean annual and ≤ 15 °C mean July temperatures.

The habitat requirements of the species present also indicate a significant environmental change. Bark beetles completely disappear. Species present include barren-ground inhabitants, such *Oreodytes laevis* which is often associated with silty waters of glacial melt streams and pools, where individuals are found covered in glacial silt (Larson, 1975). In the Yukon, Alberta and British Columbia it is found on barren substrates in cool waters at the edge of, or adjacent to, large lakes (Larson, 1997). *Elaphrus lapponicus lapponicus* lives near cold waters in areas where short vegetation and mosses grow. Today it is collected in sunny habitats, although small scattered conifers are sometimes present (Goulet, 1983). Also, remains of vegetation consist of sparse, twiggy plant matter. These factors indicate that the coniferous forest had vanished from the area to be replaced by a barren pro-glacial landscape with sparse, short vegetation and cool, barren streams and pools.

5.4: $\delta^{18}\text{O}$ and $\delta^2\text{H}$ analyses of chitin and cellulose:

Results of isotopic analyses are shown in Table 5.3 and plotted in Figures 5.5 and 5.6.

Horizon	Chitin $\delta^2\text{H}$ (‰ \pm 3.4)	Cellulose $\delta^2\text{H}$ (‰ \pm 4.1)	Chitin $\delta^{18}\text{O}$ (‰ \pm 0.62)	Cellulose $\delta^{18}\text{O}$ (‰ \pm 0.45)
0	-63.9	-129.8	16.29	25.58
1.6	-92.9	-110.9	14.30	29.95
1.7	-89.6 \pm 4.3*	-118.7	13.47 \pm 1.76*	28.84
1.8	-115.9	-111.8	12.81	29.51
1.9	-99.9	-110.3	12.69	30.82
2.0	-79.8	-104.5	12.65	26.47
2.1	-109.2	-118.4 \pm 4.2*	6.51	31.15 \pm 0.82**
2.2	-100.6	-106.8	11.96	26.84

Table 5.3: Isotopic results from Gardena samples. For samples marked with * n=2, ** n=4 and for all others n=1. Results are expressed in parts per thousand with respect to SMOW.

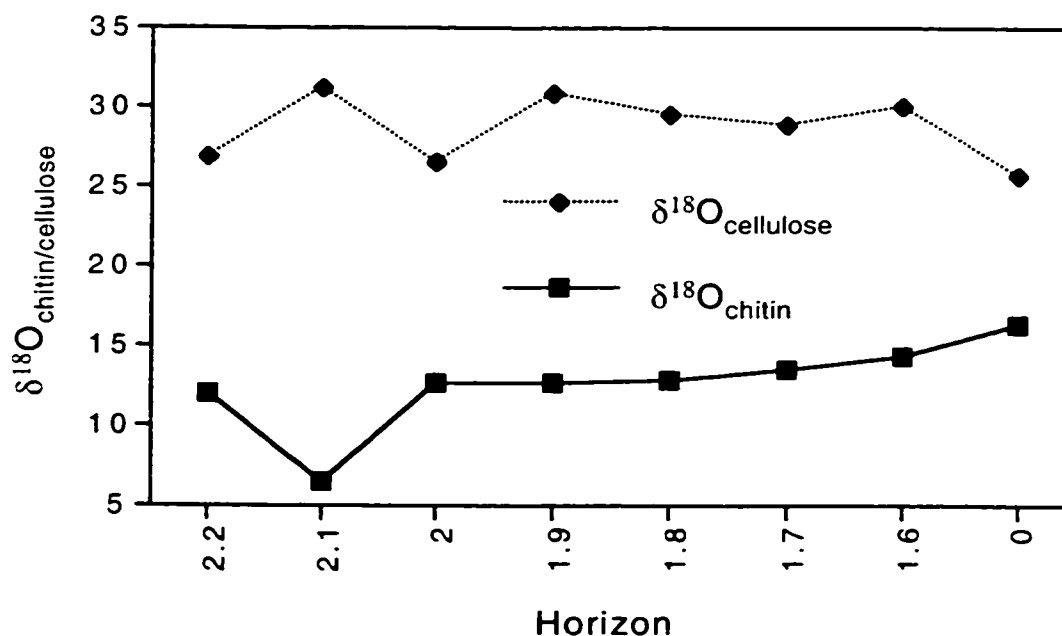


Figure 5.5: $\delta^{18}\text{O}$ analyses for chitin and cellulose.

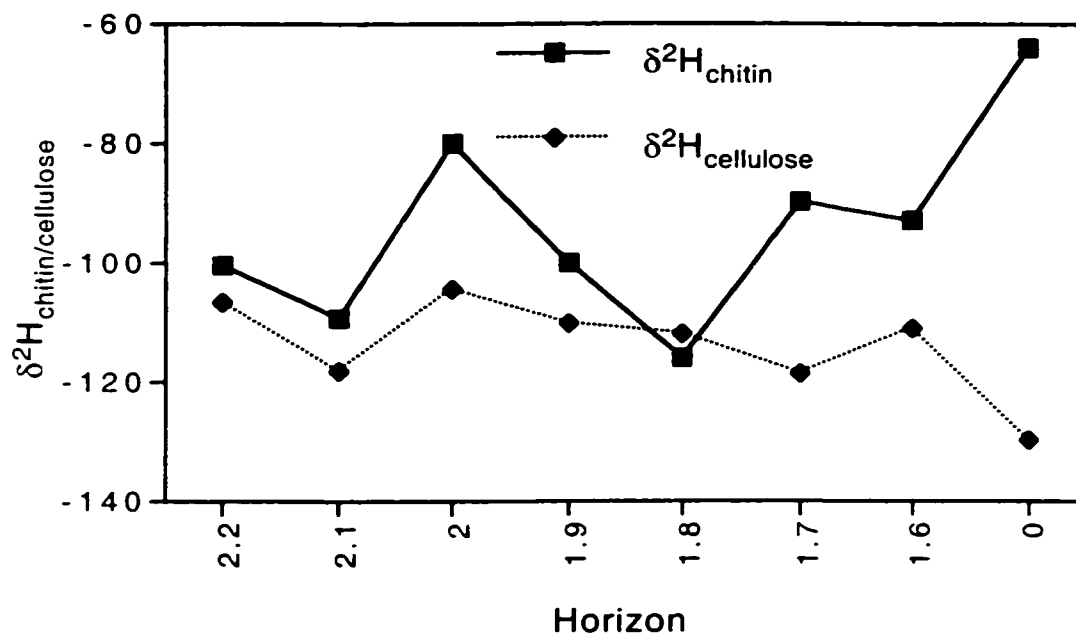


Figure 5.6: $\delta^2\text{H}$ analyses for chitin and cellulose.

Values for $\delta^{18}\text{O}_{\text{chitin}}$ are depleted with respect to $\delta^{18}\text{O}_{\text{cellulose}}$ by an average of $16.06 \pm 4.33\text{‰}$. This is a much greater depletion than was encountered in modern samples in Chapter 4, where the depletion was $3.94 \pm 1.44\text{‰}$. This divergence is due to $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ moving in opposite directions in the fossil site, with $\delta^{18}\text{O}_{\text{chitin}}$ values generally more depleted than in the modern samples and $\delta^{18}\text{O}_{\text{cellulose}}$ values generally more enriched.

Little co-variance between $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ is evident in Figure 5.5. In fact, what is striking is that the two plots describe almost opposite trends, with the $\delta^{18}\text{O}_{\text{chitin}}$ values generally increasing up the section, while the $\delta^{18}\text{O}_{\text{cellulose}}$ values have a decreasing trend. This is especially apparent at the 2.1m horizon, where chitin values dip sharply while cellulose values increase, and at the moss horizon (0m), where $\delta^{18}\text{O}_{\text{chitin}}$ increases while $\delta^{18}\text{O}_{\text{cellulose}}$ decreases.

Results for $\delta^2\text{H}_{\text{chitin}}$ are enriched with respect to $\delta^2\text{H}_{\text{cellulose}}$ by an average of $19.9 \pm 21.4\text{‰}$. This is much lower than the $53.5 \pm 15.6\text{‰}$ average

enrichment of chitin over cellulose observed in 11 sites in Chapter 4. As is the case with $\delta^{18}\text{O}$, there is considerable divergence in the two plots progressing up the section, particularly at 0m, where they move in opposite directions and at 1.9 and 1.8m where $\delta^2\text{H}_{\text{chitin}}$ is highly depleted

Although there are significant differences between the isotopic trends in chitin and cellulose, the $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$ display similar patterns, as do the $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ plots, especially when $\delta^2\text{H}_{\text{chitin}}$ values at 1.9 and 1.8m are excluded. This suggests that the processes affecting the isotopic contents of each material are consistent, even though the differences between chitin and cellulose are pronounced. A closer examination of some of these differences may reveal their origins.

At 0m opposite trends are exhibited by the chitin and cellulose isotopic values. There, $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$ are depleted 3.50 and 18.1‰ respectively when compared to the average of the other seven levels; $\delta^{18}\text{O}_{\text{chitin}}$ is enriched 4.23‰ in comparison to the average of the other seven points; while $\delta^2\text{H}_{\text{chitin}}$ is enriched 34.4‰ when compared to the average of the other levels. Taxonomic analysis shows that by this point the temperature had cooled significantly, relative to lower horizons, and the environment had changed from a boreal forest to open ground with little, if any, arboreal content. If the drop in cellulose isotopic values is due to precipitation becoming depleted as a result of a cooling environment, then the relative enrichment of chitin over cellulose is entirely an effect of other environmental influences on the insects. This would have to be significant, since any enrichment would have to compensate for a presumed depletion in the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of food materials.

Miller *et al.* (1988) found that $\delta^2\text{H}_{\text{chitin}}$ from 34 North American locales became more enriched over $\delta^2\text{H}_{\text{environmental water}}$ at higher-latitude sites relative to lower-latitude ones. He said this may be due to the fact that beetles in colder environments are only active during the warmer months, meaning they are selectively exposed to enriched summer precipitation and reflect its isotopic content, rather than a more integrated annual signal. This was demonstrated in Chapter 4 (Figure 4.19), where a gradient of $-2.1\text{‰}/^\circ\text{C}$ was observed in $\Delta^2\text{H}_{\text{chitin-environmental water}}$

Although no similar effect was noted in $\delta^{18}\text{O}_{\text{chitin}}$, it is possible that it was invisible in the smaller data set.

This effect could result in dramatic enrichments: Whereas Epstein *et al.* (1976) found that in 25 North American sites $\delta^2\text{H}_{\text{cellulose}}$ was depleted with respect to $\delta^2\text{H}_{\text{environmental water}}$ by an average of 22‰, Miller *et al.* (1988) found enrichments of $\delta^2\text{H}_{\text{chitin}}$ over $\delta^2\text{H}_{\text{environmental water}}$ of 13 to 60‰ in high-latitude sites, leading to the possibility of a total enrichment of $\delta^2\text{H}_{\text{chitin}}$ over $\delta^2\text{H}_{\text{cellulose}}$ of 80‰. Clearly, enrichments of this magnitude are not exhibited here, although these figures serve to illustrate how important this effect could be.

At 2.1m $\delta^{18}\text{O}_{\text{chitin}}$, $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ decline abruptly, while $\delta^{18}\text{O}_{\text{cellulose}}$ increases. Depletions observed in the three declining isotopes could be attributed to the influence of lighter precipitation or other environmental water, perhaps associated with a decrease in temperature. If that is the case, the increase in $\delta^{18}\text{O}_{\text{cellulose}}$ is puzzling. Looking to the taxonomic analysis gives a possible clue. At 2.1m the specific content indicates a climatic warming. Perhaps what happened at this level was the opposite of what occurred at 0m: the insects were active for a longer period each year, due to the warmer environment, and therefore incorporated the signature of isotopically lighter early- and late-season environmental water into their chitin. This would result in a counter-intuitive drop in $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ as temperature increased, coupled with increases in $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$ if precipitation became isotopically heavier. It is unclear why no increase in $\delta^2\text{H}_{\text{cellulose}}$ is apparent. The observed depletion is small, in comparison to adjacent points, so it is possible it is just an anomaly, or that the temperature increase was coupled with a decrease in humidity rather than enrichment of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in precipitation, which would enrich $\delta^{18}\text{O}_{\text{cellulose}}$ preferentially.

Explanation of the sharp drop in $\delta^2\text{H}_{\text{chitin}}$ at 1.9 and 1.8m is more difficult, since no similar decrease is observed in any of the other isotopic analyses. It is possible that some unknown environmental variable was the cause.

5.5: Calculation of environmental parameters:

To facilitate the interpretation of isotope results from this site various environmental parameters were inferred using previously-developed methodologies. Temperatures (Table 5.4 and Figure 5.7) for the eight horizons were estimated from the taxonomic content. This was accomplished by comparing the geographic ranges of the insect assemblages from each level with the isothermal contour maps in the Climatic Atlas-Canada (Anonymous, 1984).

Horizon	Temperature (°C)
0	-7
1.6	-5
1.7	0
1.8	0
1.9	0
2.0	-5
2.1	1.5
2.2	-2

Table 5.4: Annual temperatures for the various horizons estimated from the taxonomic content.

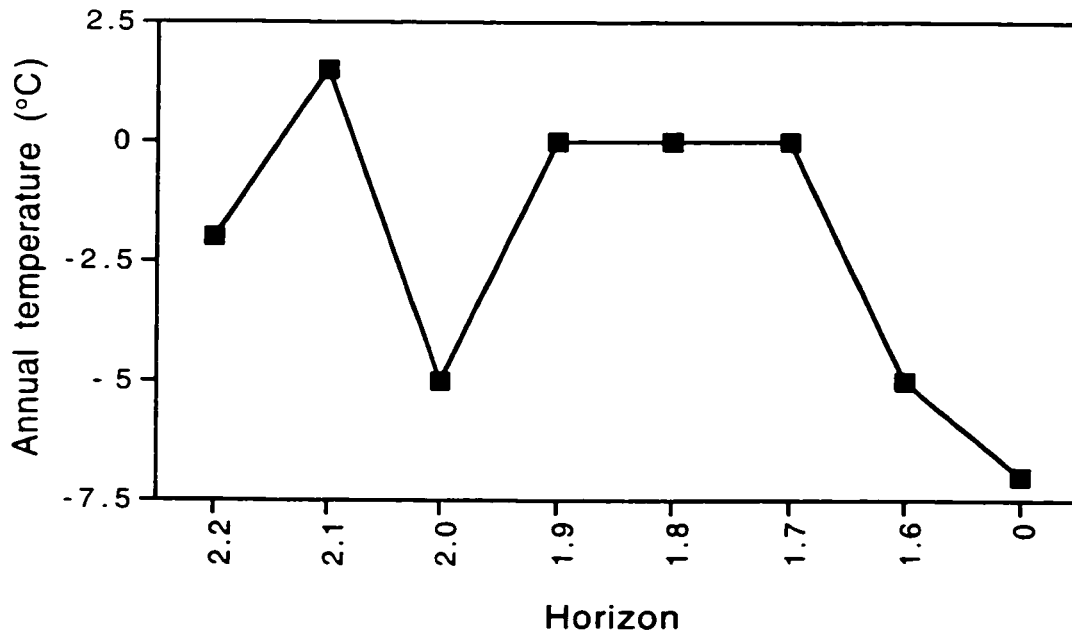


Figure 5.7: Estimated annual temperatures for the eight horizons.

Relative humidity and $\delta^{18}\text{O}_{\text{environmental water}}$ were calculated from $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$, and $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$ for each horizon. This was accomplished using the approach discussed in Section 4.4 for chitin and a similar method from Edwards and Fritz (1986) for cellulose. Constants and equations for cellulose are:

$$\frac{1000 + \delta^{18}\text{O}_{\text{cellulose}}}{1000 + \delta^{18}\text{O}_{\text{environmental water}}} = {}^{18}\alpha_n {}^{18}\alpha_e {}^{18}\alpha_k - {}^{18}\alpha_n ({}^{18}\alpha_e {}^{18}\alpha_k - 1)h \quad (5.1)$$

Where ${}^{18}\alpha_n = 1.0282$, ${}^{18}\alpha_e = 1.0095$ and ${}^{18}\alpha_k = 1.0260$.

$$\frac{1000 + \delta^2\text{H}_{\text{cellulose}}}{1000 + \delta^2\text{H}_{\text{environmental water}}} = {}^2\alpha_n {}^2\alpha_e {}^2\alpha_k - {}^2\alpha_n ({}^2\alpha_e {}^2\alpha_k - 1)h \quad (5.2)$$

Where ${}^{18}\alpha_n = 0.9530$, ${}^2\alpha_e = 1.0797$ and ${}^2\alpha_k = 1.0186$.

Values for ${}^{18}\alpha_n$, ${}^{18}\alpha_e$, ${}^{18}\alpha_k$ and ${}^2\alpha_e$ are from Edwards and Fritz (1986), ${}^{18}\alpha_k$ is the figure for leaves with dissected morphology (appropriate for a boreal environment) (Buhay *et al.* 1996) derived in Chapter 4 and ${}^2\alpha_k$ is an average from Buhay *et al.* (1996). Figures for $\delta^2\text{H}_{\text{environmental water}}$ were then calculated from $\delta^2\text{H}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ using the humidities derived as described above and the appropriate form of equation 5.2. Since calculations from $\delta^2\text{H}_{\text{chitin}}$ are temperature dependent (through ${}^2\alpha_n = (5.7 \times 10^{-5})(T^\circ\text{C})^2 - (4.5 \times 10^{-3})(T^\circ\text{C}) + 1.008$), figures were derived using the temperature values from Table 5.4. The results of these calculations are found in Tables 5.5, 5.6 and 5.7 and Figures 5.8, 5.9 and 5.10.

Horizon	$\delta^{18}\text{O}_{\text{environmental water}}$	$\delta^{18}\text{O}_{\text{environmental water}}$
	(‰) chitin-inferred	(‰) cellulose-inferred
0	-16.5	-16.4
1.6	-19.2	-14.8
1.7	-14.5	-15.8
1.8	-18.8	-14.8
1.9	-16	-15
2.0	-16.2	-12.2
2.1	-14	-16.8
2.2	-17.2	-12.8

Table 5.5: $\delta^{18}\text{O}_{\text{environmental water}}$ inferred from $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ and $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$

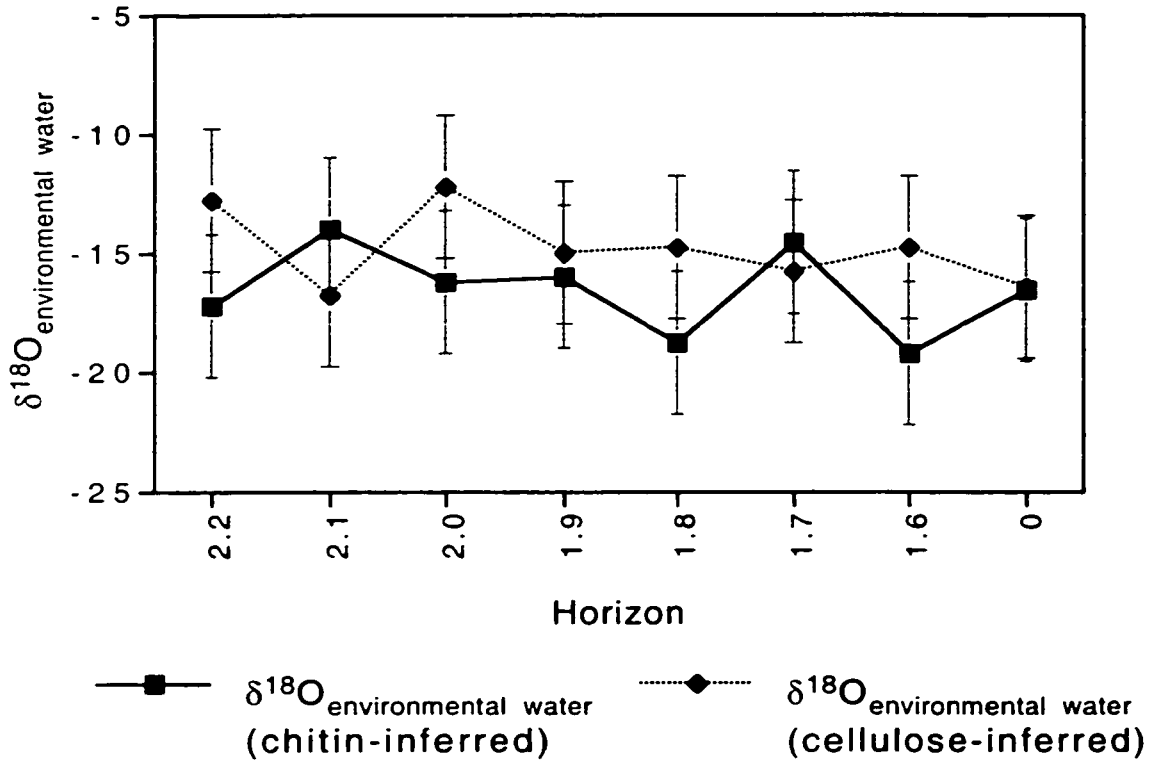


Figure 5.8: Inferred $\delta^{18}\text{O}_{\text{environmental water}}$ from Table 5.5, versus horizon, showing the correspondence between values inferred from chitin and cellulose. Error bars are $\pm 3\%$ for both chitin and cellulose-inferred values, as discussed in Section 4.4.

Horizon	$\delta^2\text{H}_{\text{environmental water}}$ (‰)	$\delta^2\text{H}_{\text{environmental water}}$ (‰)
	chitin-inferred	cellulose-inferred
0	-122.9	-121.5
1.6	-142.4	-108.9
1.7	-104.9	-116.6
1.8	-140.1	-108.5
1.9	-116.8	-110.8
2.0	-119.7	-87.6
2.1	-100.6	-123.9
2.2	-127.1	-92.2

Table 5.6: $\delta^2\text{H}_{\text{environmental water}}$ inferred from $\delta^2\text{H}_{\text{chitin}}$, $\delta^2\text{H}_{\text{cellulose}}$ and the relative humidity values from Table 5.7.

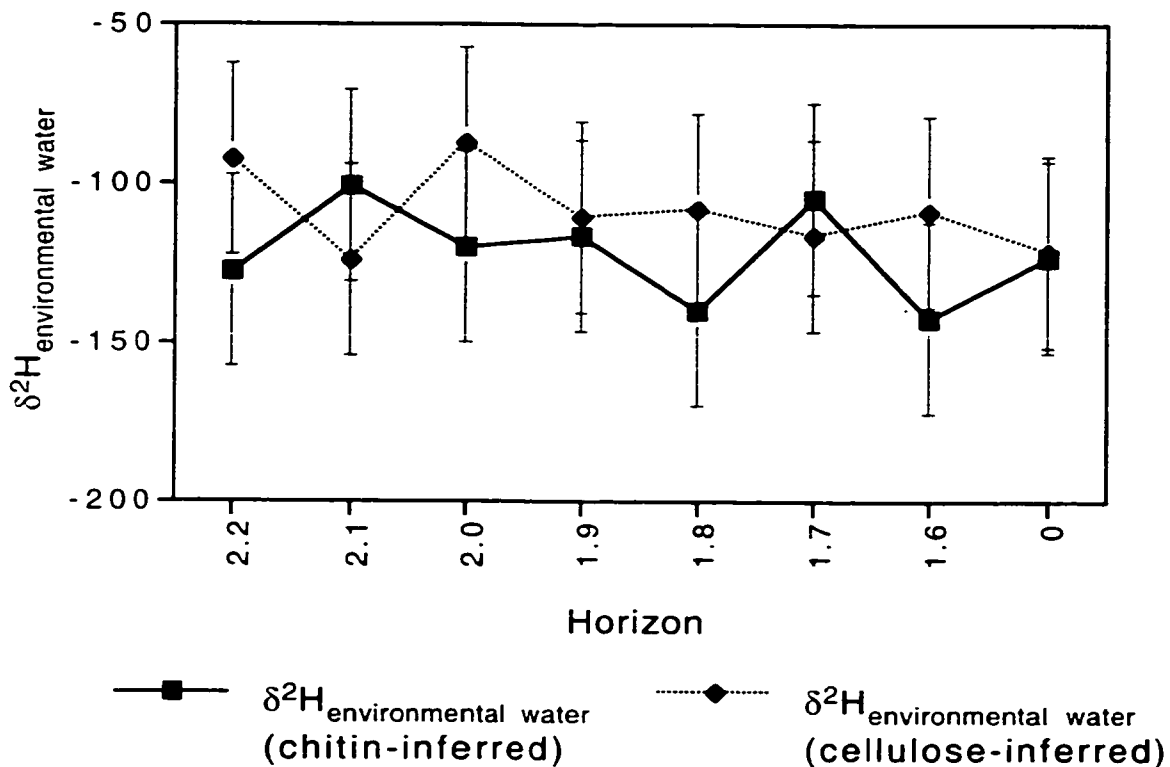


Figure 5.9: Inferred $\delta^2\text{H}_{\text{environmental water}}$ from Table 5.6, versus horizon, demonstrating that chitin and cellulose-inferred values are comparable. Error bars are ± 30 ‰ for both chitin and cellulose-inferred values, as discussed in Section 4.4.

Horizon	Relative humidity (%) chitin-inferred	Relative humidity (%) cellulose-inferred
0	76	60
1.6	75	53
1.7	91	53
1.8	80	54
1.9	89	50
2.0	87	70
2.1	111	44
2.2	87	68

Table 5.7: Relative humidities inferred from $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$, and $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^2\text{H}_{\text{cellulose}}$.

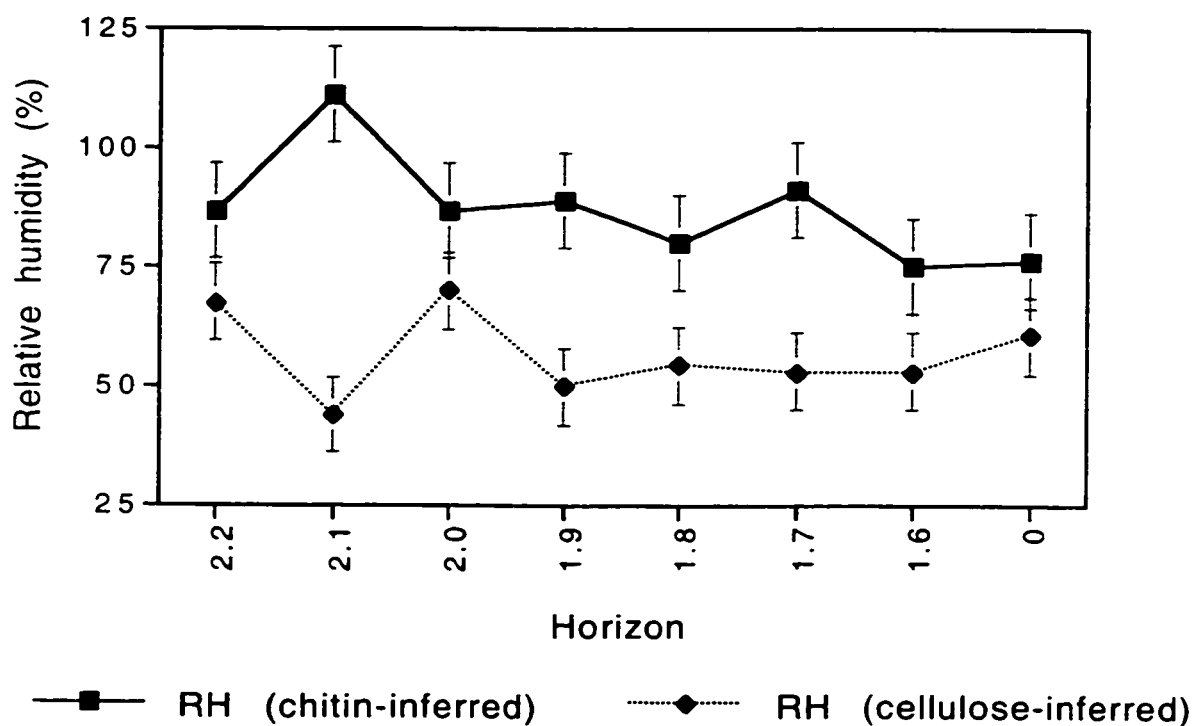


Figure 5.10: Inferred relative humidity, from Table 5.7, versus horizon. Note that values inferred from chitin are higher than those from cellulose. Error bars are $\pm 10\%$ for both chitin-inferred and $\pm 8\%$ cellulose-inferred values, as discussed in Section 4.4.

5.6: Discussion of environmental inferences:

Inferred $\delta^{18}\text{O}_{\text{environmental water}}$ (Table 5.5 and Figure 5.8) based on chitin and cellulose show reasonable correspondence, with average values of -16.6 and -14.8‰ respectively, possibly with a slight decline progressing up the section. Although there is considerable variation between the two sets of inferred data, they are similar in trend and magnitude and are comparable in the context of the probable uncertainty inherent in this approach. Modern precipitation $\delta^{18}\text{O}$ in Illinois (Chicago) is -6.90‰ (Rozanski, *et al.*, 1993). The highest inferred $\delta^{18}\text{O}_{\text{environmental water}}$ values are similar to those from southern Ontario and Quebec today, while the lowest are like those from parts of southern prairie provinces. The inferred $\delta^{18}\text{O}_{\text{environmental water}}$ values are in accord with a general circulation model (GCM) simulation of precipitation isotopes during the last glacial maximum (LGM), around 18,000 BP, in Jouzel *et al.* (1994) which predicted a depletion of about 10‰ in precipitation $\delta^{18}\text{O}$ values, compared to the present.

$\delta^2\text{H}_{\text{environmental water}}$ (Table 5.6 and Figure 5.9) inferred from chitin and cellulose also show a reasonable correlation in trend and magnitude within the probable limits of this methodology. Average values for $\delta^2\text{H}_{\text{environmental water}}$ inferred from chitin and cellulose are -121.8 and -108.7‰ respectively, with a slight decline progressing up the section, similar to that observed with $\delta^{18}\text{O}_{\text{environmental water}}$. Modern precipitation $\delta^2\text{H}$ for Chicago is -50.0‰ (Rozanski, *et al.*, 1993). As was the case with oxygen, $\delta^2\text{H}_{\text{environmental water}}$ figures range from highs similar to southern Ontario to lows like those from parts of the southern prairie provinces today. The inferred $\delta^2\text{H}_{\text{environmental water}}$ values are in accord with the GCM simulation of precipitation isotopes during the LGM in Jouzel *et al.* (1994) which predicted a depletion of about 80‰ in precipitation $\delta^2\text{H}$ values compared to the present.

The inferred $\delta^2\text{H}_{\text{environmental water}}$ values are in sharp contrast to much more enriched figures for the northern midwestern United States derived from carbon-bonded hydrogen in wood cellulose (analyzed from cellulose nitrate) by Yapp and Epstein (1977). These were -36‰ from a 22,000 BP site in Ohio, -44‰ from a 20,000 BP site in Indiana and -35‰ from a 18,500 BP site in Kentucky. The authors invoked a variety of

explanations for the enrichment over modern values: a reduced winter-summer temperature gradient; oceanic cooling; enriched ocean surface waters; variation in the relative proportions of winter and summer precipitation; and a positive shift in oceanic water vapour. It should be noted that these $\delta^{2}\text{H}_{\text{environmental water}}$ values were inferred based on the assumption that that $\delta^{2}\text{H}_{\text{environmental water}}$ is enriched by a fixed 20‰ over $\delta^{2}\text{H}_{\text{cellulose nitrate}}$. It is likely that this simple model failed to account for other fractionating influences, such as evapotranspiration.

Construction of a $\delta^{18}\text{O}$, $\delta^{2}\text{H}$ diagram (Figure 5.11), as in Chapter 4, provides a diagrammatic representation of the relationship between oxygen and hydrogen isotopes in chitin and cellulose and the global meteoric water line (GMWL). Use of a GMWL configured such that $\delta^{2}\text{H} = 8\delta^{18}\text{O} + 10$ is appropriate based on a GCM simulation of precipitation isotopes during the LGM in Jouzel *et al.* (1994). It is clear that the leaf water inferred from chitin is less evaporatively enriched than that inferred from cellulose. Reasons for this are dealt with below in the discussion of humidity inferences, however, the result of this effect is that leaf water $\delta^{18}\text{O}$ and $\delta^{2}\text{H}$ inferred from chitin and cellulose describe an evaporation line (line A in Figure 5.11). This line is very similar to one (line B in Figure 5.11) constructed from leaf water and environmental water isotopic values inferred from cellulose. Note that both evaporation lines are constructed assuming no *f*-factor damping.

Extrapolating evaporation line A back to the GMWL provides $\delta^{18}\text{O}$ and $\delta^{2}\text{H}$ values for unevaporated source water. These are $\delta^{18}\text{O} = -16.6\text{‰}$ and $\delta^{2}\text{H} = -122.6\text{‰}$, which are very close to the average environmental water values of $\delta^{18}\text{O} = -16.6\text{‰}$ and $\delta^{2}\text{H} = -121.8\text{‰}$ inferred from chitin using the chitin model and similar to the average values of $\delta^{18}\text{O} = -14.8\text{‰}$ and $\delta^{2}\text{H} = -108.7\text{‰}$ inferred from cellulose using the Edwards and Fritz (1986) model. Although this discussion deals with the data from the site as a group, producing average values, the same methodology could be applied to the levels individually. The similarity of the three sets of inferred environmental water isotopic values suggests that analysis of $\delta^{18}\text{O}$ and $\delta^{2}\text{H}$ for chitin and cellulose from a site, along with an knowledge of α_n for both isotopes and a temperature estimate may provide an alternate means of inferring $\delta^{18}\text{O}$ and $\delta^{2}\text{H}$ of environmental water. This calculation is also sensitive to temperature,

through ${}^2\alpha_n$ of chitin: Re-evaluating the leaf water $\delta^2\text{H}$ calculation and extrapolation of environmental water isotopic values using fixed temperatures of -5, -2.2 (the average for all levels), 0 and 5°C for determining ${}^2\alpha_n$ yields isotope-temperature gradients of 0.9‰/°C for $\delta^{18}\text{O}_{\text{environmental water}}$ and 7.3‰/°C for $\delta^2\text{H}_{\text{environmental water}}$.

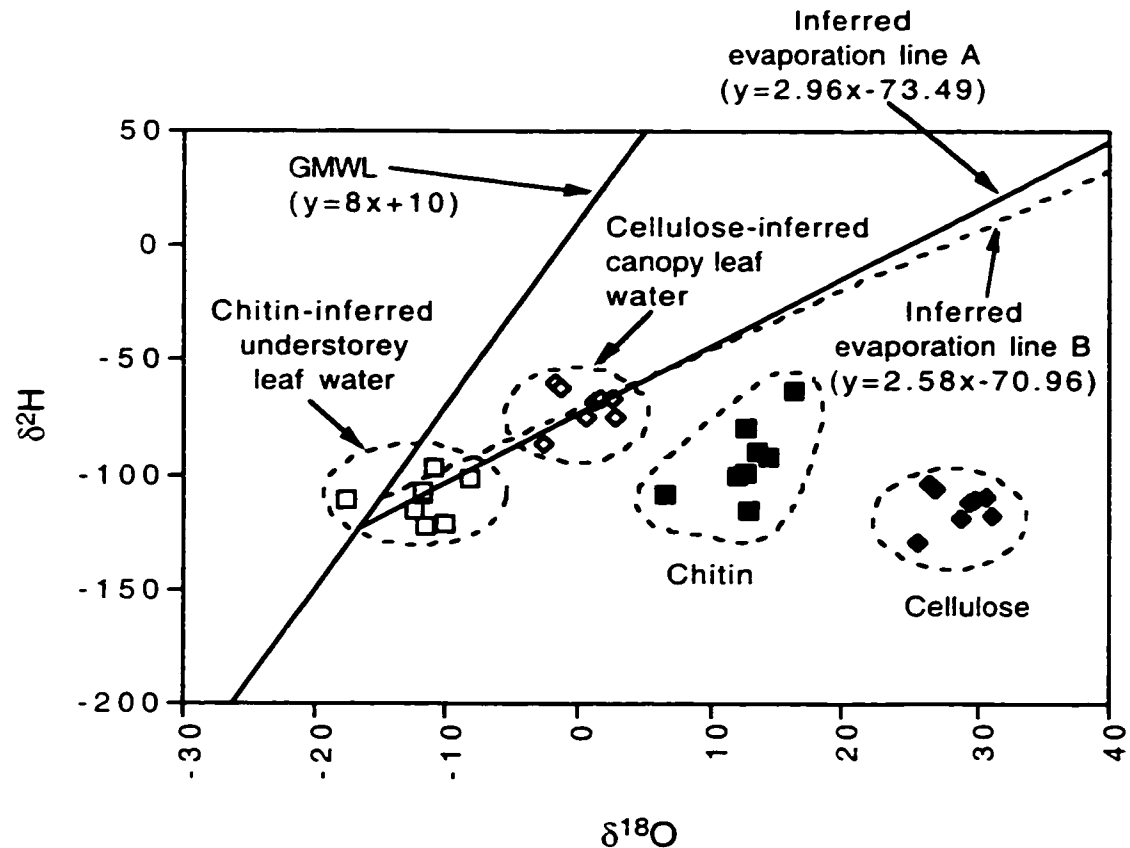


Figure 5.11: Plot of $\delta^2\text{H}_{\text{chitin}}$ versus $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{cellulose}}$ versus $\delta^{18}\text{O}_{\text{cellulose}}$ along with $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for leaf water derived from those values. Leaf-water figures were calculated from the chitin and cellulose models, based on $(1000 + \delta_{\text{sample}})/(1000 + \delta_{\text{leaf water}}) = \alpha_n$. Evaporation line A is a regression of all inferred leaf water values, while evaporation line B is a regression of cellulose-inferred leaf water and environmental water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ derived from cellulose (Tables 5.5 and 5.6). Note the similarity between the two evaporation lines.

Applying a temperature-dependent hydrologic effect to the $^{18}\alpha_n$ of chitin (Figure 5.12), as discussed in Chapter 4 ($^{18}\alpha_n = (7.1 \times 10^{-6})(T^\circ\text{C})^2 - (5.6 \times 10^{-4})(T^\circ\text{C}) + 1.025$), produces a tighter cluster of chitin-inferred leaf water points. The evaporation line (evaporation line C) derived from these values and the cellulose-inferred leaf water data produces a regression much closer to evaporation line B. Extrapolating evaporation line C to the GMWL provides inferred environmental water isotopic values of $\delta^{18}\text{O} = -15.6\text{‰}$ and $\delta^2\text{H} = -114.8\text{‰}$, which are also similar to the average figures for environmental water inferred from chitin and cellulose using their respective models with no $\delta^{18}\text{O}_{\text{chitin}}$ temperature dependency. Furthermore, average leaf water $\delta^{18}\text{O}$ and $\delta^2\text{H}$ inferred from chitin, $\delta^{18}\text{O} = -13.6\text{‰}$ and $\delta^2\text{H} = -110.5\text{‰}$, are very close to average inferred environmental water isotopic values, particularly those derived from cellulose, suggesting that, with temperature dependencies applied to the $^{18}\alpha_n$ and $^2\alpha_n$ of chitin, there is little or no evaporative enrichment apparent in this site. However, as was said in Chapter 4, application of a temperature effect to the $^{18}\alpha_n$ of chitin is very speculative because of the lack of evidence that it exists.

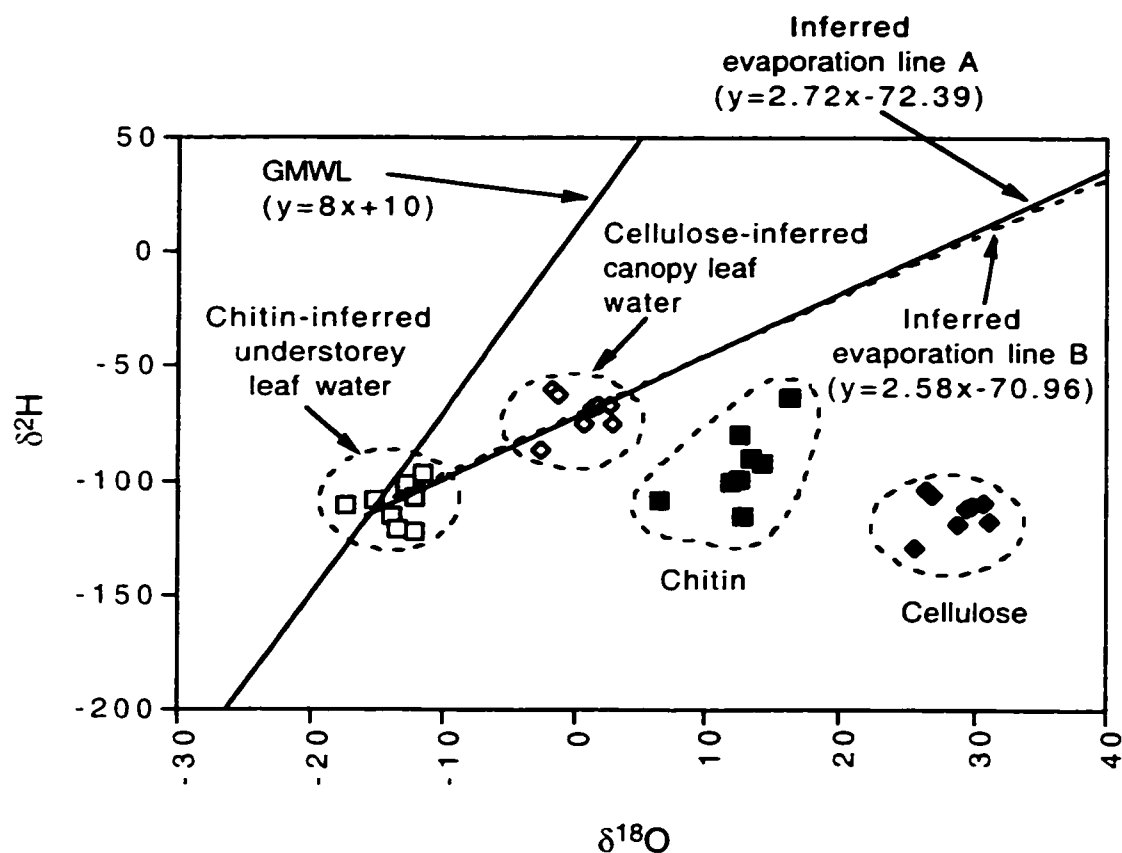


Figure 5.12: Revision Figure 5.11 with a temperature dependency applied to chitin-inferred $\delta^{18}\text{O}_{\text{leaf water}}$ values. Evaporation line C is a regression of all inferred leaf water values, while evaporation line B is a regression of cellulose-inferred leaf water and environmental water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ inferred from cellulose (Tables 5.5 and 5.6). Note that the evaporation lines are even closer together than was the case in Figure 5.11 and that the chitin-inferred leaf-water values form a tighter cluster close to the GMWL.

Humidity results (Table 5.9 and Figure 5.10) present an intriguing problem. The chitin-derived values are much higher than those inferred from cellulose by an average of $30 \pm 17\%$. This effect may result from an environmental difference between the insects and the trees from which

the cellulose is derived. Trees transpire from leaves relatively high in the air where humidity would be lower and wind speeds higher, contributing to increased evaporation and isotopic enrichment. The insects, living either on the ground or close to it, would exist in an environment protected from wind, with higher humidities, deep beneath the forest canopy. Indeed, Edwards, *et al.* (1985) speculate that free-air humidity is likely lower than that within a forest canopy.

Insects derive their isotopic composition from their food sources which are, directly or indirectly, plants: Directly in the case of herbivores, and indirectly in the case of predators or scavengers. While bark beetles would be exposed to an arboreal food source which reflects the humidity of the general area, most other beetles would receive their isotopic composition from low-lying plants living in the forest floor environment. Therefore, in a mixed assemblage, such as that represented by this site, much of the insect chitin could be more reflective of forest floor, rather than atmospheric, wind and humidity conditions. This could explain the relatively greater depletion of $\delta^{18}\text{O}_{\text{chitin}}$ in the fossil samples than in modern ones, since modern specimens discussed in Chapter 4 were predominantly tree feeders and would, therefore, reflect higher evapotranspiration. The same explanation may apply to $\delta^2\text{H}_{\text{chitin}}$, which is less enriched with respect to cellulose than in modern samples, although humidity-related effects are less pronounced in hydrogen.

This effect could also cause the increase in $\delta^{18}\text{O}_{\text{chitin}}$ at the 0m level. At this point the climate apparently cooled, as discussed in the taxonomic section, but the area was also clear of trees. It is possible that the open landscape conditions exposed the low-lying food plants of the insects to higher wind and lower humidity conditions than had previously been the case, resulting in greater evapotranspiration and isotopic enrichment, despite the cooler conditions. A similar effect is not evident in $\delta^{18}\text{O}_{\text{cellulose}}$ because the plants used for these samples were exposed to similar conditions throughout the section, so the transition to open ground is marked by a depletion, reflecting that in precipitation, rather than increased evapotranspirative enrichment. The humidity effects at 0m may have been accentuated in $\delta^{18}\text{O}_{\text{chitin}}$ by the influence of seasonality, discussed earlier.

The observed increase in $\delta^2\text{H}_{\text{chitin}}$ at 0m is more difficult to explain, since $\delta^2\text{H}$ is less influenced by evaporation than $\delta^{18}\text{O}$. Seasonality likely played a part, since this seems to have more of an influence on $\delta^2\text{H}_{\text{chitin}}$ than $\delta^{18}\text{O}_{\text{chitin}}$. A change in evapotranspirative conditions probably also played a role.

Humidity effects may have also accentuated a seasonal influence at 2.1m. While humidity above 100% are clearly impossible, the humidity peak indicated by chitin (Figure 5.10) at this horizon may reflect a moister forest-floor environment, linked to the climatic warming indicated by the taxonomic analysis. The drop in humidity indicated by cellulose isotopes (Figure 5.10) reflects the evapotranspirative gradient that existed between tree tops and forest floor. Why humidity greater than 100% is produced by the model is uncertain. This could result from scatter around the GMWL and, given the uncertainties inherent in this model, a value in the high 90% range is plausible.

Since the isotopic content of meteoric water can be related to temperature, the inferred $\delta^{18}\text{O}_{\text{environmental water}}$ and $\delta^2\text{H}_{\text{environmental water}}$ figures were used to calculate temperatures (Figures 5.13 and 5.14). These were derived from $\delta^{18}\text{O}_{\text{environmental water}}$ using the 0.58‰/°C gradient for the International Atomic Energy Agency/World Meteorological Organization network from Rozanski, *et al.* (1993) and modern figures of $\delta^{18}\text{O}_{\text{environmental water}} = -6.90\text{‰}$ and temperature = 10.2°C for Chicago (Rozanski, *et al.*, 1993) as starting points, resulting in the expression $\delta^{18}\text{O}_{\text{environmental water}} = 0.58T^{\circ}\text{C} - 12.8$; and $\delta^2\text{H}_{\text{environmental water}}$ using the equation $\delta^2\text{H}_{\text{environmental water}} = 4.2T^{\circ}\text{C} - 119.7$ from Chapter 4. Temperatures derived from $\delta^2\text{H}_{\text{environmental water}}$ are similar to those calculated earlier (Table 5.4 and Figure 5.7) and are suggestive of south-central to central Canada today. Those inferred from $\delta^{18}\text{O}_{\text{environmental water}}$ are lower than the other two: more like those in modern-day Canada from the central prairie provinces to the southern Northwest Territories.

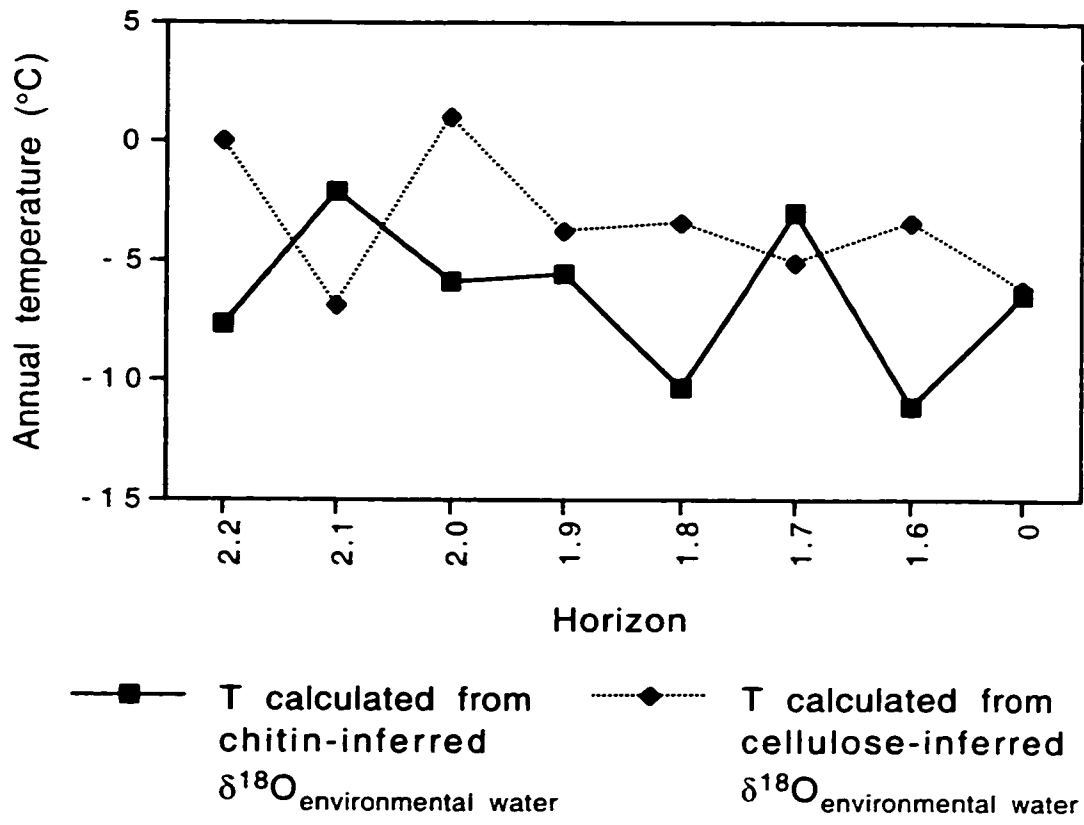


Figure 5.13: Annual temperature derived from $\delta^{18}\text{O}_{\text{environmental water}}$ in Table 5.6 using $\delta^{18}\text{O}_{\text{environmental water}} = 0.58T^{\circ}\text{C} - 12.8$.

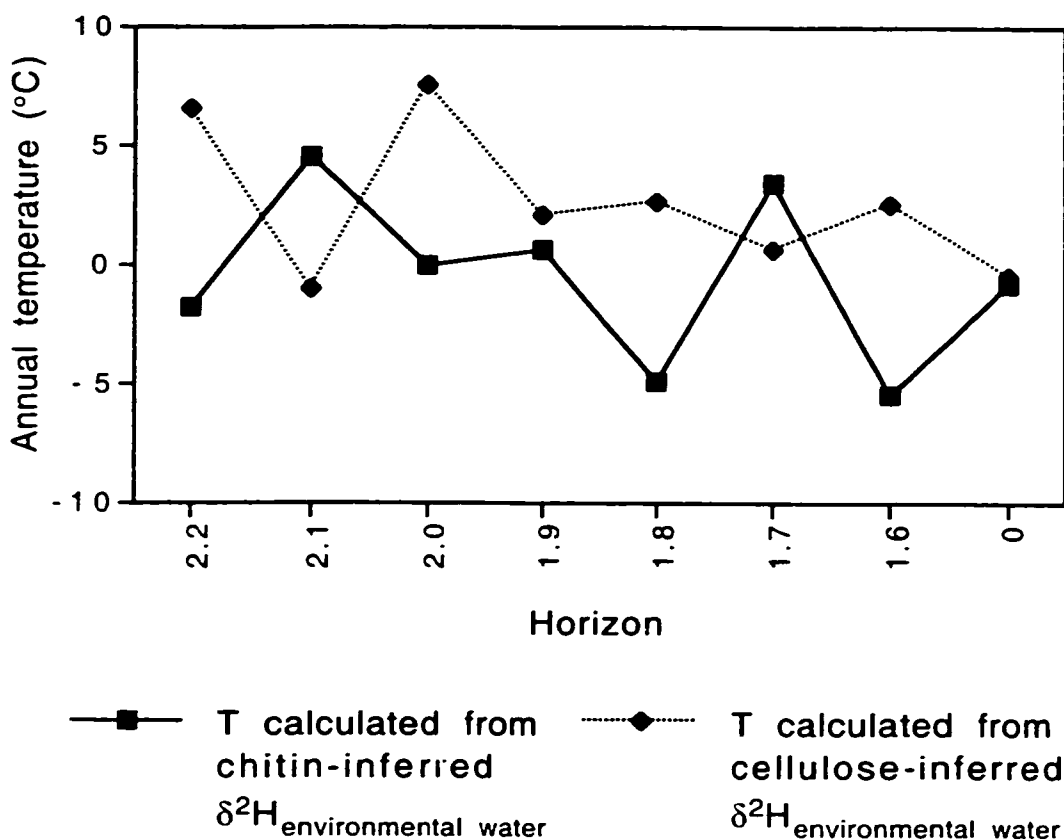


Figure 5.14: Annual temperature derived from $\delta^2\text{H}_{\text{environmental water}}$ in Table 5.7 using $\delta^2\text{H}_{\text{environmental water}} = 4.2T^\circ\text{C} - 119.7$.

Since the chitin and cellulose models produce $\delta^{18}\text{O}_{\text{environmental water}}$ values, and temperatures can be estimated from the insect taxonomic content, a $\delta^{18}\text{O}$ /Temperature relationship can be constructed for this site (Figures 5.15 and 5.16). Using a GCM simulation of precipitation isotopes in North America during the LGM, Jouzel *et al.* (1994) calculated slopes for $\delta^{18}\text{O}$ versus temperature relationships of 0.55 for the LGM (spatial relationship) and 0.37 for the time period from the LGM to the present (temporal relationship). The authors note that errors associated with calculating the temporal slope could be quite large because only two points, those for the LGM and the present, were used. Figure 5.15 shows that the chitin-inferred $\delta^{18}\text{O}$ /T relationship forms a pattern which is in

general agreement with both the LGM spatial and temporal slopes. The trend in Figure 5.15 is also similar to the modern $0.58\text{‰}/^{\circ}\text{C}$ $\delta^{18}\text{O}_{\text{precipitation}}$ gradient for the International Atomic Energy Agency/World Meteorological Organization network (Rozanski, *et al.*, 1993) supporting the GCM result that modern and LGM spatial $\delta^{18}\text{O}/T$ slopes are similar (Jouzel *et al.*, 1994). The cellulose-inferred $\delta^{18}\text{O}/T$ relationship (Figure 5.16) shows no discernible trend. Nonetheless, these results are encouraging because, in the case of the chitin-inferred $\delta^{18}\text{O}/T$ relationship at least, the similarity between the measured and modeled relationships suggests that natural processes are similar to those used in the GCM, and a more extensive fossil suite may provide additional corroboration.

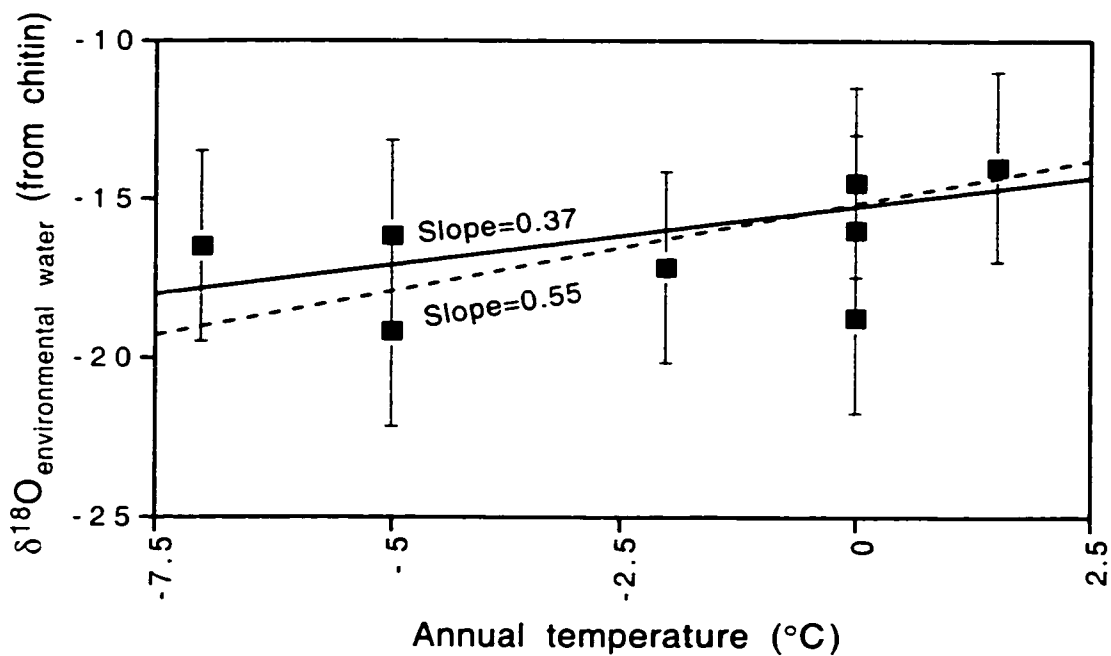


Figure 5.15: $\delta^{18}\text{O}_{\text{environmental water}}$ inferred from chitin versus annual temperature inferred from insect taxonomic content. Lines are not regressions of the data but are constructed using GCM generated temporal (LGM to present) and spatial (LGM) slopes for North America of 0.37 and 0.55 respectively (Jouzel *et al.*, 1994). Error bars are $\pm 3\text{‰}$.

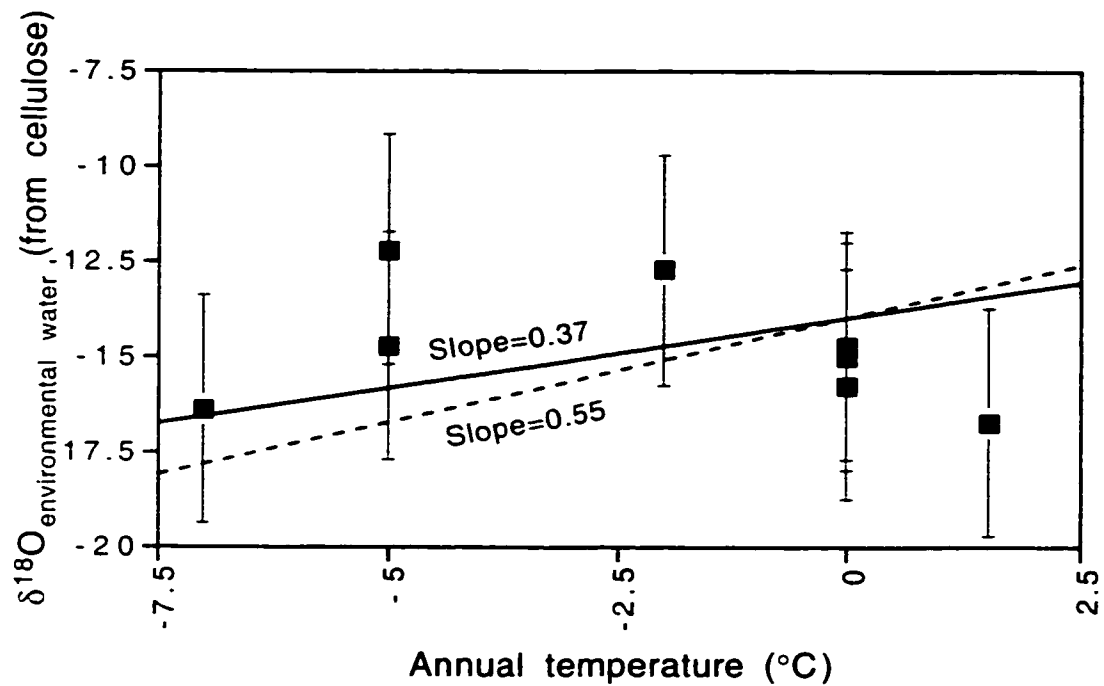


Figure 5.16: $\delta^{18}\text{O}_{\text{environmental water}}$ inferred from cellulose versus annual temperature inferred from insect taxonomic content. Lines are not regressions of the data but are constructed using GCM generated temporal (LGM to present) and spatial (LGM) slopes for North America of 0.37 and 0.55 respectively (Jouzel *et al.*, 1994). Error bars are $\pm 3\text{‰}$.

5.7: Consolidated analysis of the Gardena site:

A general climatic picture emerges from the isotopic and paleoentomological analyses: At about 26,000 BP the area was occupied by a boreal forest with annual temperatures of approximately -2°C , typical of central Canada today. Following this, temperature may have increased briefly, to about 2°C , then probably declined gradually, in concert with glacial advance, until by 19,700 BP the area was devoid of trees and host to an open environment with cold annual temperatures of $\leq 5^{\circ}\text{C}$, typical of northern-boreal Canada today. It is difficult to be more specific with this profile, since it is based on several different methodologies for estimating temperature. However, it is clear that the climate was much colder than it is currently in Illinois, and that it became cooler progressing up the section.

Humidity generally decreases progressing up the section. Chitin-indicated values are generally higher, indicating wet conditions, while those indicated by cellulose are drier, suggesting a moderate to dry climate. The two points in the succession that seem to show the most deviation from the average climate for the period are at 2.1 and 0m.

At 2.1m the taxonomic content indicates a warming, which is partially supported by some of the other inferred temperatures. This is accompanied by an increase in humidity, as indicated by chitin isotopes, and a drop in humidity inferred from cellulose isotopes. Given this contradiction, it is likely that a climatic warming at this interval was accompanied by a vegetational change, perhaps an increased density of forest cover, which provided a more sheltered, thereby warmer and more humid, environment for the insects. This may have been linked to a decrease in humidity in the atmosphere as a whole, perhaps accompanied by an increase in wind speed, which resulted in increased evapotranspiration in the trees.

At 0m, a decrease in temperature was accompanied by a significant environmental change from forested to open ground. This change is marked by a decrease in humidity, as indicated by chitin, and an increase, as indicated by cellulose. Deforestation probably caused these inferred humidities to move in opposite directions by leaving the insects and their food plants more exposed to ambient conditions of humidity and wind than they had been previously. Furthermore, humidity estimates

derived from chitin and cellulose are closer together than at any other point in the section, suggesting that chitin isotopic values there are more indicative of general free-air humidity than at other horizons.

Based on the inferred humidity pattern for 2.1 and 0m it appears that the spread between humidity values based on chitin and cellulose isotopes is indicative of the degree of forest cover, with the chitin-inferred humidity representing ground-level values and the cellulose-inferred values being indicative of humidity in the general area. There is some noise in this signal, caused by the overprinting of effects relating to changing taxonomic content of the insect assemblage, variations in average wind speed and changing environmental water isotopic content. However, the effect appears to be pronounced enough to penetrate these other signals under conditions where a change is significant.

Inferred environmental water $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values are somewhat higher than that which would be expected in modern areas with climates similar to those indicated by the inferred temperatures and other environmental indicators from this site. This is not surprising, since the compression of climatic zones, which would be expected in close proximity to a continental ice sheet (Levesque, *et al.*, 1997), would likely produce a latitudinal precipitation isotopic gradient different from that which is present today.

The foregoing climatic interpretation is partially supported by palynological, plant macrofossil and other paleoentomological evidence. Morgan (1987) suggested a severe climatic gradient at the ice margin and reported fossil insects indicating annual temperatures of about -1 to 0°C and midboreal conditions at 27,000 to 21,000 year old sites in central Indiana and Illinois, including Gardena. Analysis by that author of the upper horizon (0m) at Gardena and a nearby site in central Illinois, dating from 20,500 BP, indicated a cold, open and treeless environment, with mean annual temperatures in the range of -8 to -7°C.

King (1979) found the Robein Silt, which occupies the lower 30 cm of the section (about 26,000 BP), was dominated by pine and spruce pollen, indicating a stable coniferous forest. The moss horizon (0m, 19,700 BP) contained spruce and pine pollen, with a high spruce percentage indicating that spruce was more abundant than pine. The presence of arboreal pollen, however, does not necessarily mean that

trees were present at the site, only that they were close enough for the pollen to be transported there. Given the compression of climate zones, and extreme climatic gradient, in front of an ice sheet (Levesque, *et al.*, 1997) it is not difficult to envision a situation where a treeless environment was in fairly close proximity to a forest.

Miller (1979) analyzed moss from the 0m horizon and found that all samples represented extant species which grow on wet substrates in the vicinity of stagnant or flowing water. He concluded that the environment at the time of deposition was probably one of relatively open ground, although a higher-density forest could have been nearby.

Chapter 6:

Recommendations for further research:

While the work in this thesis demonstrates a methodology for using the isotopic content of fossil insect chitin to infer paleoclimatological information, several aspects of this research merit further exploration.

Pyrolysis and equilibration provide good results and allow oxygen and hydrogen isotopes to be examined in the same sample, however, tedious preparation is required and there is the possibility that minute fossil samples will be lost during processing. Furthermore, pyrolysis is probably not the best technique for very small samples because of fractionation effects in both oxygen and hydrogen. To overcome these limitations different approaches are required to compensate for exchangeable hydrogen and measure isotopic content. Both equilibration and nitration are labour-intensive and, possibly, sample-destroying procedures, so it would be worthwhile to run experiments to determine whether these procedures could be eliminated. Chitin is purified using laboratory deionized water and NaOH and it is possible that much of the exchangeable hydrogen is equilibrated with the preparation water during this processing. Since laboratory water probably varies relatively little in $\delta^2\text{H}$ over time, it should be possible to quantify its effect on routinely prepared chitin and calibrate for this, possibly using a standard prepared with each batch of samples. In order to increase accuracy with very small samples, continuous-flow isotope-ratio mass spectrometry could be used, eliminating any size-dependent fractionation effects inherent in the pyrolysis procedure.

While the chitin models for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ are good starting points for further research, a better estimate of the precision is desirable. To this end, it would be useful to collect insect and wood samples from localities where precipitation isotope and meteorological data is available. This would be possible near weather stations in locations where records of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in precipitation have been kept. This data would also allow more precise determination of fractionation factors, at least for those sites sampled, because such things as temperature and wind speed at those exact locations would be known.

Although there is no evidence for a temperature-dependent hydrologic effect on $\delta^{18}\text{O}_{\text{chitin}}$ like the one demonstrated for $\delta^2\text{H}_{\text{chitin}}$, a larger $\delta^{18}\text{O}_{\text{chitin}}$ data set than was available for this research would be useful to either confirm that no such effect exists or show that it is present after all. To this end, collection and $\delta^{18}\text{O}_{\text{chitin}}$ analysis of further insect samples from the range of environments used in the $\delta^2\text{H}_{\text{chitin}}$ discussion would be a valuable exercise.

In order to verify some of the effects observed in the fossil site, it would be worthwhile to target specific modern environments for further study. In particular, a survey of moist environments, collecting both chitin and cellulose, would help to fine-tune the humidity dependence of the chitin model. Also, a study of arboreal and non-arboreal chitin and cellulose from a variety of environments would serve to confirm whether the disparity between chitin and cellulose-inferred humidity in the fossil site is indicative of the degree of forest cover and, perhaps, provide some insight into quantifying this effect. It may also provide an indication of the accuracy of evaporation lines inferred from combined chitin and cellulose isotopic analyses. On a more general level, a larger chitin and cellulose $\delta^{18}\text{O}$ data set and more cellulose $\delta^2\text{H}$ linked to chitin would allow refinement of the model.

Chitin isotopic analyses of other fossil sites which have been studied by workers in other paleoenvironmental disciplines would aid in confirming its utility in elucidating ancient climates. This could, perhaps, indicate ways in which chitin provides additional information about environments for which there are no modern analogues. Carrying this to its logical conclusion, isotopic analysis of insects in amber would provide the ultimate test of our ability to use chitin to push climatic insights far into the past.

Although this study has concentrated on oxygen and hydrogen isotopes, chitin also contains carbon and nitrogen. Limited work has been done on isotopes of these two elements in chitin in the past (Miller (1984) for carbon and Schimmelmann and DeNiro (1986b) for carbon and nitrogen), but there is great scope for further work, especially since elemental analyzer technology allows simple and rapid analyses of both of these elements.

Chapter 7:

Conclusions:

While chitin isotopic analysis has received little attention in the past, it is clear from this study that it can provide useful paleoenvironmental information. There is a clear link between $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ and the isotopic content of precipitation, as well as demonstrable influences from other environmental factors such as temperature and relative humidity. A link between the isotopic content of chitin in herbivorous insects and that of cellulose is also apparent, showing that isotopic labeling of precipitation can be traced through the food chain.

The effects of environmental factors on the isotopic content of chitin can be quantified in such a way as to permit the formulation of a $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$ model with predictive capabilities. Application of this model to fossil material yields isotopic and environmental information that is in good agreement with cellulose isotopic analysis as well as GCM results and data from traditional paleoentomological, palynological and plant macrofossil analysis. It is best when employed along with an adjunct technique, such as paleoentomological, palynological or cellulose isotopic analysis because insect seasonality is incorporated in the model as temperature dependence in the biological fractionation of $\delta^2\text{H}_{\text{chitin}}$. Employment of another technique allows the estimation of temperature for use in the chitin model, facilitating the inference of $\delta^{18}\text{O}_{\text{environmental water}}$, $\delta^2\text{H}_{\text{environmental water}}$ and relative humidity from $\delta^{18}\text{O}_{\text{chitin}}$ and $\delta^2\text{H}_{\text{chitin}}$.

Combination with isotopic analysis of cellulose presents the opportunity to derive even more information than is available from each technique on its own. The apparent difference in the humidity regimes influencing insects and plants allows the formulation of an evaporation line, based on leaf water values inferred from chitin and cellulose, which, when extrapolated to the GMWL, yields an environmental water isotopic composition for the site. Application of this approach to the Gardena fossil site in this thesis produced environmental water isotopic values similar to those inferred from chitin and cellulose using their respective models. Furthermore, the difference between humidities inferred from chitin and cellulose appears to reflect the gradient between understorey (chitin) and canopy (cellulose) evapotranspirative

conditions and may provide some indication of forest cover if it can be calibrated with modern studies.

Taxonomic paleoentomological analysis is a natural adjunct to chitin isotopic study since insects are separated by hand for both techniques and it is relatively straightforward to identify the fossils before processing for isotopic analysis. Ideally, study of a fossil site would employ chitin and cellulose isotopic analyses, along with taxonomic paleoentomology, as was done with the Gardena site, to extract the maximum amount of information.

While the utility of chitin isotopic analysis is clear, it is also apparent that more research is required before it can be employed as a routine technique. Sample preparation and handling, especially of sparse fossil material, is difficult and would benefit from the application of new technologies and some investigation into whether procedures could be simplified. Also, the model used to relate the isotopic composition chitin to the environment of its formation could be improved with further research by targeting specific localities with particular ecological characteristics or good suites of environmental and isotopic data.

In summary, isotopic analysis of chitin presents a virtually untouched field for study with many new avenues to explore. The research in this thesis has laid groundwork for future work, but further efforts are required for this method to achieve its full potential.

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Appendix 1: Analytical values from pyrolysis and equilibration experiments.

$\delta^2\text{H}_{\text{true}}$ (‰ SMOW) Experiment 2	$\delta^2\text{H}_{\text{measured}}$ (‰ SMOW) Experiment 2	$\delta^2\text{H}_{\text{true}}$ (‰ SMOW) Experiment 3	$\delta^2\text{H}_{\text{measured}}$ (‰ SMOW) Experiment 3	$\delta^{18}\text{O}_{\text{true}}$ (‰ SMOW) Experiment 3	$\delta^{18}\text{O}_{\text{measured}}$ (‰ PDB) Experiment 3
21.45	20.97	21.45	23.02	32	-10.39
21.45	23.391	-42.12	-35.73	32	-10.38
-42.12	-40.94	-117.87	-135.21	27	-16.69
-42.12	-37.24	-176.48	-188.95	27	-17.33
-42.12	-41.44			18.6	-23.39
-117.87	-104.19			18.6	-23.78
-117.87	-102.47				
-117.87	-105.34				
-176.48	-160.84				
-176.48	-158.43				

Table A: True and measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values used to calibrate Experiment 2 and 3 devices.

$\delta^2\text{H}_{\text{water}}$ (‰ SMOW)	$\delta^2\text{H}_{\text{equilibrated chitin}}$ (‰ SMOW)
21.4	-11.96
-42.1	-22.53
-42.1	-16.33
-73.5	-18.85
-73.5	-24.02
-73.5	-20.83
-117.9	-27.74
-117.9	-28.89
-176.5	-42.18

Table B: $\delta^2\text{H}$ values for equilibration water and equilibrated chitin used in Section 3.

Appendix 2: Chitin isolation procedure (Miller, 1984).

- i) Soak insects in 10% NaOH solution at 100 °C, replacing solution every 24 hours. Continue until fresh solution dissolves no-new material (usually 48 to 72 hours). This step dissolves all non-chitinous material.
- ii) Decant NaOH solution and wash, successively, in 9:1; 3:1; 1:1; and 1:3 ethanol:water solutions.
- iii) Wash in de-ionized water.
- iv) Wash in 0.4% HCl. This step neutralizes any remaining NaOH.
- v) Soak in de-ionized water for 24 hours.
- vi) Freeze-dry if working with dry chitin.
- vii) Store wet if equilibration is planned.

Appendix 3: Cellulose isolation procedure (Green, 1963).

- i) Grind wood and plant material to powder.
- ii) Soak in 2:1 benzene:ethanol for 48 hours then decant. This step and step iii remove lipids, resins and tannin.
- iii) Soak in acetone for 24 hours, decant and air-dry.
- iv) Add de-ionized water to sample vessels, then cover and place in water bath at 70 °C.
- v) Add 0.5 ml of glacial CH_3COOH and 0.5 gm NaCl_2 to each sample every hour (seven or eight additions). This step removes lignin.
- vi) Filter and wash with de-ionized water.
- vii) Soak in 17% NaOH for 45 minutes. This step removes xylan, mannan and other polysaccharides.
- viii) Filter and wash with de-ionized water.
- ix) Soak in 10% CH_3COOH for 15 minutes to neutralize NaOH .
- x) Filter and wash until there is no odour.
- xi) Decant and freeze.
- xii) Freeze-dry.

Appendix 4: Fossil-insect extraction procedure (Morgan and Morgan, 1980).

- i) Wash sediment sample through 300-micron sieve, placing the portion retained on the sieve in a shallow plastic container.
- ii) Drain and knead kerosene into the sample. The kerosene causes insects and seeds to float when water is added.
- iii) Drain excess kerosene.
- iv) Fill sample vessel with cold water, making sure to thoroughly agitate the material.
- v) Let sit for a few minutes to allow suspended material to settle.
- vi) Carefully drain water through 300-micron sieve, ensuring that only the floating material is removed from the container.
- vii) Wash material retained on sieve with soap, water and ethanol, washing it into a beaker with the alcohol. This step removes kerosene.
- viii) Repeat steps iv through vii until no more material floats.
- ix) Store sample in ethanol until ready for sorting.