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**Departament de Producció Vegetal i Ciència forestal**

# Reconstruction of climatic and crop conditions in the past based on the isotope signature of archaeobotanical remains

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A IRENE
cuando era niña

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## Good Use Right

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# Summary

## Reconstruction of climatic and crop conditions in the past based on the isotope signature of archaeobotanical remains

The onset and progressive development of agriculture during the last 10,000 years has modified radically the social and demographic structure of human groups, as well as their interaction with the environment. In fact, the adoption of agriculture is probably the first example of reciprocal interaction between the environment and the humanity. Thus, reconstructing the environmental conditions (either climatic or anthropogenic) that characterised this process is of great interest in order to know their potential causes, as well as to understand the long-term effects of agriculture economy on the environment. The general objective of this Thesis is the development of new tools addressed to the reconstruction of climatic and crop conditions during the origins and spread of agriculture in Prehistory. The proposed methodology is based on the study of plant remains (wood charcoal and charred seeds) routinely recovered during archaeological excavations, with special emphasis on the use of stable isotopes as palaeoenvironmental indicators. The work can be divided into two main blocks. The first attempts to get a picture of the climatic context in which evolved agriculture, while the second one is centred on the specific growing conditions of cultivated crops.

The objective of the first bock is to obtain a methodology allowing the quantification of aridity changes in the past from the stable isotope analysis of wood charcoal from forest species. This requires a previous calibration with modern material, as well as the assessment of the effect of carbonisation on the stable isotope signature of wood. The first chapter characterises the climatic signature in carbon isotope composition ($\delta^{13}C$) of wood in Mediterranean conditions, using *Pinus halepensis* Mill. and *Quercus ilex* L. as reference species, finding that in both species $\delta^{13}C$ is mostly related to precipitation. The second chapter studies the relationship between climate and oxygen isotope composition ($\delta^{18}O$) for *P. Halepensis*, as well as the differences in the isotopic signature ($\delta^{13}C$ and $\delta^{18}O$) in the two major components of wood: cellulose and lignin. We found that the climatic signal of $\delta^{13}C$ is equally present in cellulose and lignin, whereas for $\delta^{18}O$ cellulose extraction is necessary to obtain a climatic signal, which prevents its application in carbonised material. Finally, the third chapter shows how carbonisation, although affecting significantly the $\delta^{13}C$ of wood, does not remove the original climatic signal, which can be recovered by means of a simple correction, taking carbon concentration as a reference variable. Using this methodology, we reconstructed aridity changes in the Cinca and Segre valleys (Ebro Depression) during the last 4,000 years, finding greater water availability in the last millennia than in present.

The second block describes different aspects of crop conditions in prehistoric agriculture, combining $\delta^{13}C$ analyses with morphometrical variables of cultivated species, mostly cereals. Chapter four constitutes a critical review of the isotopic data available for archaeobotanical remains, proposing new approaches in order to maximise the information retrieved from these data. It shows how the combined analysis of crop and forest species, as well as the comparison among different crops, may help to detect human-driven changes in crop water availability. Chapter five develops a new methodology to estimate grain weight in cereals cultivated in the past, from charred grain dimensions, and considering the effect of carbonisation. This allows, for the first time, a direct comparison between archaeobotanical data and current agronomic studies. Finally, Chapter six combines data obtained using the methods described in the three former chapters to study the potential causes of the observed grain weight changes in Cinca and Segre valleys during the last 4,000 years.

# Resum

## Reconstrucció de les condicions climàtiques i de conreu en el passat a partir de l'anàlisi isotòpica de restes arqueobotàniques

L'aparició i progressiu desenvolupament de l'agricultura durant els darrers 10.000 anys ha alterat radicalment l'estructura social i poblacional dels grups humans, així com la seva relació amb l'entorn. De fet, l'adopció de l'agricultura pot constituir el primer exemple en el temps d'interacció recíproca entre el medi ambient i la humanitat. Per tant, la reconstrucció de les condicions ambientals (d'origen climàtic o degudes a l'acció humana) que van caracteritzar aquest procés és de gran interès per tractar de conèixer-ne les causes, així com per entendre els efectes a llarg termini que ha tingut l'economia agrícola en el medi. L'objectiu general de la tesi és desenvolupar noves eines destinades a reconstrucció de les condicions climàtiques i de conreu durant l'origen i dispersió de l'agricultura a la prehistòria. La metodologia que es proposa està basada en l'estudi de les restes vegetals (fustes i llavors carbonitzades) que són recuperades rutinàriament en excavacions arqueològiques, posant especial èmfasi en l'ús dels isòtops estables com a indicadors paleoambientals. El treball es pot dividir en dos grans blocs. El primer pretén configurar l'entorn climàtic en el que va evolucionar l'agricultura, en tant que el segon se centra en la caracterització de les condicions específiques de creixement de les espècies conreades.

El primer bloc té com a objectiu la posada a punt d'una metodologia que permeti quantificar els canvis d'aridesa en el passat, a partir de l'anàlisi isotòpica de carbons d'espècies forestals. Això requereix una calibració prèvia amb material actual, així com una valoració de l'efecte de la carbonització sobre la signatura isotòpica de la fusta. Al primer capítol, es va caracteritzar la signatura climàtica present a la composició isotòpica de carboni ($\delta^{13}C$) de la fusta en condicions mediterrànies, fent servir *Pinus halepensis* Mill. i *Quercus ilex* L. Com a espècies de referència, trobant que en totes dues espècies aquesta es podia relacionar sobretot amb la precipitació. Al segon capítol, es va estudiar la relació entre el clima i la composició isotòpica d'oxigen ($\delta^{18}O$) per *P. halepensis*, així com les diferències en la signatura isotòpica ($\delta^{13}C$ i $\delta^{18}O$) present als dos components fonamentals de la fusta: cel·lulosa i lignina. Es va trobar que la component climàtica de la $\delta^{13}C$ és igualment present a cel·lulosa i lignina, en tant que per la $\delta^{18}O$ l'extracció de cel·lulosa és necessària per tal d'obtenir informació climàtica, fet que impedeix la seva aplicació en material arqueològic. Finalment, al tercer capítol es va poder demostrar com la carbonització, tot i alterar significativament la $\delta^{13}C$ de la fusta, no n'elimina la component climàtica original, que pot recuperar-se mitjançant una senzilla correcció, que fa servir el contingut de carboni com a variable de referència. Amb aquesta metodologia es reconstrueixen els canvis d'aridesa que han tingut lloc a les valls del Cinca i del Segre (Depressió de l'Ebre) durant els darrers 10.000 anys, trobant una major disponibilitat hídrica al llarg dels darrers mil·lenis que no pas en el present.

El segon bloc aborda la descripció de diferents vessants de les condicions de conreu a l'agricultura prehistòrica, combinant l'anàlisi de la $\delta^{13}C$ amb mesures morfomètriques en llavors d'espècies conreades, fonamentalment cereals. El capítol quart constitueix una revisió crítica de les dades existent sobre isòtops estables en restes arqueobotàniques, proposant noves aproximacions per tal de maximitzar la informació que se'n pot derivar. Es mostra com la combinació d'anàlisis en espècies conreades i forestals, així com la comparació entre diferents conreus, pot ajudar a detectar canvis en la disponibilitat hídrica dels conreus, fruit de l'acció humana.El cinquè capítol desenvolupa una nova metodologia que permet estimar el pes del gra dels cereals conreats en el passat, a partir de les seves dimensions, tot considerant l'efecte de la carbonització. Això permet, per primer cop, establir una lligam directe entre les dades obtingudes a partir de restes arqueobotàniques i els estudis agronòmics. Finalment, el capítol sisè combina dades obtingudes a partir dels mètodes descrits en els tres capítols anteriors per resoldre les possibles causes dels canvis observats en la mida del gra als jaciments les valls del Cinca i del Segre durant els darrers 4.000 anys.

# Resumen

## Reconstrucción de las condiciones climáticas y de cultivo en el pasado a partir del análisis isotópico de restos arqueobotánicos

La aparición y progresivo desarrollo de la agricultura durante los últimos 10.000 años ha alterado radicalmente la estructura social y poblacional de los grupos humanos, así como su relación con el entorno. De hecho, la adopción de la agricultura puede que constituya el primer ejemplo en el tiempo de interacción recíproca entre el medio ambiente y la humanidad. De ahí que reconstruir las condiciones ambientales (de origen climático o debidas a la acción humana) que caracterizaron este proceso sea de gran interés para tratar de conocer sus posibles causas, así como para entender los efectos a largo plazo que ha tenido la economía agrícola en el medio. El objetivo general de esta tesis es el desarrollo de nuevas herramientas destinadas a reconstruir las condiciones climáticas y de cultivo durante el origen y dispersión de la agricultura en la prehistoria. La metodología propuesta se basa en el estudio de los restos vegetales (maderas y semillas carbonizadas) que son recuperados rutinariamente en excavaciones arqueológicas, poniendo especial énfasis en el empleo de los isótopos estables como indicadores paleoambientales. El trabajo se puede dividir en dos grandes bloques. El primero pretende configurar el entorno climático en el que evolucionó la agricultura, mientras que el segundo se centra en caracterizar las condiciones específicas de crecimiento de las especies cultivadas.

El primer bloque tiene como objeto la puesta a punto de una metodología que permita cuantificar los cambios de aridez en el pasado, partiendo del análisis isotópico de carbones de especies forestales. Esto requiere una calibración previa con material actual, así como una valoración del efecto de la carbonización en la señal isotópica de la madera. En el primer capítulo, se caracterizó la señal climática presente en la composición isotópica de carbono ($\delta^{13}C$) de la madera en condiciones mediterráneas, empleando *Pinus halepensis* Mill. y *Quercus ilex* L. como especies de referencia, encontrando que en ambas especies ésta se podía relacionar fundamentalmente con la precipitación. En el segundo capítulo, se estudió la relación entre el clima y la composición isotópica de oxígeno ($\delta^{18}O$) para *P. halepensis*, así como las diferencias en la señal isotópica ($\delta^{13}C$ y $\delta^{18}O$) presente en los dos componentes principales de la madera: celulosa y lignina. Se encontró que la señal climática de la $\delta^{13}C$ es igualmente presente en celulosa y lignina, mientras que para la $\delta^{18}O$ la extracción de celulosa es necesaria para obtener una información climática, lo que impide su aplicación en material carbonizado. Finalmente, en un tercer capítulo se demostró cómo la carbonización, si bien afecta significativamente la $\delta^{13}C$ de la madera, no elimina la señal climática original, que puede recuperarse mediante una simple corrección, incluyendo el contenido de carbono como variable de referencia. Mediante este metodología se reconstruyen los cambios de aridez ocurridos en los valles del Cinca y del Segre (Depresión del Ebro) durante los últimos 4.000 años, observando una mayor disponibilidad hídrica en los últimos milenios en relación con el presente.

El segundo bloque se centra en describir diferentes aspectos de las condiciones de cultivo en la agricultura prehistórica, combinando el análisis de la $\delta^{13}C$ con medidas morfométricas en semillas de especies cultivadas, fundamentalmente cereales. El capítulo cuarto constituye una revisión crítica de la información isotópica existente sobre restos arqueobotánicos, proponiendo nuevos enfoques destinados a maximizar la información extraíble a partir de estos datos. Se muestra cómo la combinación de análisis en especies cultivadas y forestales, así como la comparación entre cultivos, puede ayudar a detectar cambios en la disponibilidad hídrica de los cultivos debidos a la acción humana. El capítulo quinto desarrolla una nueva metodología que permite estimar el peso del grano en los cereales cultivados en el pasado, a partir de sus dimensiones, y considerando el efecto de la carbonización. Esto permite, por primera vez, una comparación directa entre los datos obtenidos a partir de restos arqueobotánicos y la bibliografía agronómica. Finalmente, el capítulo sexto combina datos obtenidos mediante los métodos descritos en los tres capítulos anteriores para resolver las posibles causas de los cambios en el tamaño del grano observados en los valles del Cinca y del Segre durante los últimos 4.000 años.

# General Introduction

*“Kung Tsé preguntó a Yeu:*
*¿Sabes en qué consiste la verdadera ciencia? En conocer que se sabe lo que realmente se sabe, y que se ignora lo que en verdad se ignora.”*

Kung Tsé. *Lun Yu*

# I. Overview

During the Holocene, agriculture has been adopted in many parts of the world as independent processes: till now, several archaeological evidences have been found for plant domestication in the Near East, Ethiopia, sub-Saharan Africa, China, Mesoamerica and South America. In particular, western agriculture, based on cereals (wheat and barley) and legumes (pea and lentil), started in an area of the Near East known as the “Fertile Crescent”, comprising the planes of Mesopotamia, the deserts of Syria and Palestine, moreover to some mountain areas of Anatolia. Around the world, agriculture appeared during the last 10,000 years, a few millennia after the retreat of Pleistocene ice, spreading during what has been the most stable warm period in almost half a million years. Thus, the origins and subsequent expansion of agriculture cannot be isolated from the particular environmental conditions in which evolved. A similar reasoning lead V. Gordon Childe to develop, within the concept of “Neolithic revolution”, the theory that climate changes altered the environment in such a way that forced hunters-gatherers to modify their life-style (Childe 1954). Other authors, while embracing the major role of climate, emphasised the importance of economic changes and cultural and social evolution (Cauvin 1994, further references in Buxó 1997 and Harlan 1975). It has been also argued that demographic pressure might have forced this change (Binford 1981; Cohen 1981), but archaeological evidences suggest that increased population was a consequence rather than a cause of the adoption of agriculture (Harlan 1975; Harris and Hillman 1989).

As quoted by Harlan (1975) in his “no-model” model for the onset of agriculture, every attempt to provide a general answer for agricultural origins has generated evidence against it, and the most probable explanation is that plant cultivation began in different areas for different reasons. What seems undoubtful is that climate have had always a major role in the success of early farming communities, given that, even in today’s most advanced agricultural exploitations, climate is the main factor determining crop yields. On the other hand, the spread of agriculture caused changes in landscape and environment without precedent in the history of human-environment interaction. Agriculture can be associated not only to current deforestation and erosion in many areas in the world, but also might have altered significantly the course of climate, initially at the local scale, but ultimately at a global dimension. Thus, the adoption and spread of agriculture might be considered the first example of reciprocal interaction between climate and the humanity. In such context, reconstructing the environmental conditions prevailing in prehistoric agriculture is of great relevance. On the one hand, it may help to understand the context in which agriculture developed, and on the other, it may be useful to attain information regarding the long-term environmental effects of agriculture in the past.

Past global climate changes during the Holocene have been well defined through a variety of climate proxies (Committee on abrupt climate change 2002; Folland *et al.* 2001). However, there is a need to concentrate efforts at the local scale, in order to attain a clearer picture of the specific climatic conditions faced by early farmers. This is particularly true for precipitation, as it shows a considerable spatial heterogeneity (Magny *et al.* 2003). The general objective of this Thesis is to develop new tools addressed to 1) reconstruct the specific climatic conditions (mostly in terms of water availability) in which ancient agriculture was developed, providing information at a local scale, and 2) to determine the use of different agronomic practices addressed to improve yields and crop water availability. The methodology proposed is based on the study of carbonised plant remains (wood charcoal and charred seeds) that are routinely recovered in the course of archaeological excavations. Although emphasis is mainly put on the use of stable isotopes as palaeoenviromental indicators, the Thesis takes also advantage of the information provided by several biomorphometric parameters. The work can be structured in two main blocks:

### BLOCK 1. Reconstruction of climate

The general objective is to develop a new methodology in order to quantify past aridity changes through the analysis of stable isotopes in wood charcoal. This block includes 3 chapters:

- **Chapter 1** compares the effect to climate and site variables on carbon isotope composition and radial growth for two Mediterranean trees, *Pinus halepensis* Mill. and *Quercus ilex* L.
- In **Chapter 2** the climatic relationships of *P. halepensis* are further studied, considering both carbon and oxygen isotopes, in wood and its two main components, lignin and cellulose.
- Finally, **Chapter 3** assess the effect of carbonisation on the carbon isotope composition of wood from *P. halepensis*, and presents a case study to reconstruct aridity changes during the last four millennia in the Ebro Basin from the analysis of fossil charcoal.

### BLOCK 2. Reconstruction of crop conditions

This block aims to describe different aspects of crop conditions in prehistoric agriculture, combining the analysis of carbon isotopes with morphometric measurements in archaebotanical remains of cultivated species, mostly cereals. It is also divided into three chapters:

- **Chapter 4** is a critical review of current isotopic data regarding archaeobotanical remains, proposing new ways to deal with these data, through the combination of analysis in cultivated and forest species, as well as by comparing different crops.
- In **Chapter 5** a new methodology is developed in order to obtain a quantitative estimation of cereal grain weight in the past from grain dimensions, bearing in mind the effect of carbonisation.
- Finally, **Chapter 6** combines data obtained from the methodologies described in the three former chapters to solve the possible causes of the observed changes in grain weight during the last four millennia in the Ebro Basin.

# II. Stable isotopes in plant science

## II.1 Theory and nomenclature

Isotopes are atoms of the same element that have different numbers of neutrons. Differences in the number of neutrons among the various isotopes of an element mean that the various isotopes have different masses. The superscript number to the left of an element designation is called the mass number and is the sum of the number of protons and neutrons in the isotope. For example, all isotopes of oxygen have 8 protons; however, an oxygen atom with a mass of 18 (denoted $^{18}O$) has 2 more neutrons than an oxygen atom with a mass of 16 ($^{16}O$). Isotopes can be divided into radioactive and non-radioactive. The former disintegrate spontaneously over time to form other isotopes, whereas the latter do not appear to decay to other isotopes on geologic time scales, and thus are also known as stable isotopes.

The most abundant elements in the biosphere are carbon (C), hydrogen (H), oxygen (O) and nitrogen (N), being $^{13}C$, $^{2}H$ (D), $^{18}O$ and $^{15}N$ the stable isotopes of greater interest in plant physiology. The stable isotope composition of a given sample is determined by mass spectrometry, an is usually expressed in diffential notation:

**Eq. I**

$$\delta(‰) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where $\delta$ stands for the isotopic composition, in parts per mil (‰),as referred to an standard (see Table I). R denotes the ratio of the heavy to light isotope (e.g., $^{13}C/^{12}C$), and $R_{\text{sample}}$ and $R_{\text{standard}}$ are the ratios in the sample and standard, respectively. A positive $\delta$ value means that the isotopic ratio of the sample is higher than that of the standard; a negative $\delta$ value means that the isotopic ratio of the sample is lower than that of the standard. For example, a $\delta^{13}C$ value of -28‰ means that the $^{13}C/^{12}C$ of the sample is 28 parts-per-thousand lower than the $^{13}C/^{12}C$ of the standard (Pee-Dee Belemnite limestone).

Isotopic composition of a given element varies considerably between the different pools of the biosphere. This phenomenon is called isotopic fractionation, and is determined by isotope effects occurring during the cleavage or formation of atomic bonds, as well as during other processes affected by atomic mass (e.g. diffusion). Thus, some substances are enriched in the heavier isotope, while others become depleted (i.e. lighter). We can distinguish two kinds of isotopic effects: kinetic ad thermodynamic. The former are due to differences between isotopes in the rate of a given reaction and the latter reflect divergences in the equilibrium constants of the reaction. Kinetic isotope effects of a chain of reactions are generally non-additive, whereas thermodynamic effects are additive. Since isotope effects have values usually very close to unity, they are often expressed in terms of isotopic discrimination (Δ), defined as its deviation from unity:

**Eq. II**

$$\Delta\left(‰\right)=\alpha-1=\frac{\delta_{\mathrm{r}}-\delta_{\mathrm{p}}}{1+\delta_{\mathrm{p}}/1000}$$

where $\alpha$ is the isotope effect associated with the reaction, and $\delta_r$ and $\delta_p$ stand for the isotopic composition of reactives and products, respectively. Moreover of being a more intuitive expression of the consequences of a given process, it allows an easier comparison of the results obtained by different researchers.

**Table I** Standards, notation, abundance, typical range in plants and mean analytical error of the stable isotopes most commonly used in plant physiology. Adapted from Mateo *et al.* (2004). Original data from Barbour *et al.* (2001), Ehleringer and Rundel (1988), Epstein *et al.* (1977), Farquhar *et al*. (1989) and Handley and Raven (1992).

| | **Isotope pair** | | | |
|---|---|---|---|---|
| | **$^{13}C/^{12}C$** | **$^{15}N/^{14}N$** | **$^{18}O/^{16}O$** | **$^{2}H(D)/^{1}H$** |
| Standard | PDB[b] | Air $N_2$ | SMOW[c] | SMOW[c] |
| Differential notation | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{18}O$ | $\delta D$ |
| Mean abundance (%)[a] | 1,1 | 0,37 | 0,20 | 0,015 |
| Observed range δ (‰) | -35 to -5 | -10 to +10 | +15 to +35 | -300 to +20 |
| Analytical error[d] (‰) | 0.1 | 0.2 | 0.05-0.2 | 4-7 |

[a] Abundances of the heavier isotope against the total pool of the element
[b] PDB, Pee-Dee Belemnite (limestone): already used up, replaced by secondary standards
[c] SMOW, Standard Mean Ocean Water
[d] Overall analytical precision (standard error: sample preparation + internal error of mass spectrometer)

## II.2 Carbon isotopes in plant physiology

The isotopic family of carbon includes two stable isotopes ($^{12}C$ and $^{13}C$) and several radioactive isotopes. Carbon is the most abundant element in the biosphere and, moreover, participates in virtually any of the biological reactions. Indeed, the study of carbon isotope composition ($\delta^{13}C$) provides useful information about several physiological processes and is particularly useful as an integrative measurement of plant response against the environment. On the other hand, $^{13}C$ is relatively abundant (Table I) which, along with its wide range of variation, makes its determination easier, allowing very precise results at a relatively low cost.

The range of variation of $\delta^{13}C$ in nature is greater than 100‰ (Ehleringer and Rundel 1988). However, the most usual values in the geosphere and biosphere remains between –40‰ and 0‰ (see Fig. I). Average $\delta^{13}C$ of atmospheric $CO_2$ is -8‰, although this value is becoming more negative year by year (ca. 0.02-0.03‰/year) due to the synergic effect of deforestation and the use of fossil fuels (Keeling *et al.* 1979). Moreover, there is an important seasonal variation of atmospheric $\delta^{13}C$, which is determined by the vegetation cover, being far clearer in the Northern than in the Southern hemisphere (Fig. II). Such divergence is caused by the fact that, in Northern hemisphere, vegetation is concentrated in medium to high latitudes, and thus exposed to seasonal cycles of activity, whereas Southern hemisphere vegetation is dominated by the equatorial rainforest. Atmospheric $\delta^{13}C$ variations (seasonal or interanual) should be considered when comparing species differing in their growing cycles, as well as distant samples, either in

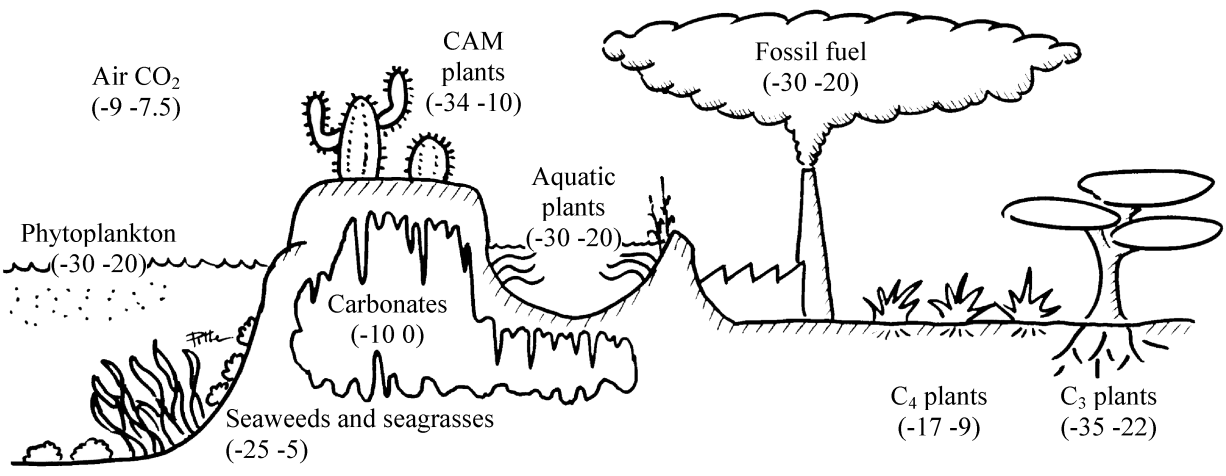


**Fig. I** Range of carbon isotopic composition ($\delta^{13}C$) within the main compartments of environment and biosphere. Redrawn from Mateo *et al.* (2004). Original data from Ehleringer and Rundell (1988) and Vogel (1993).

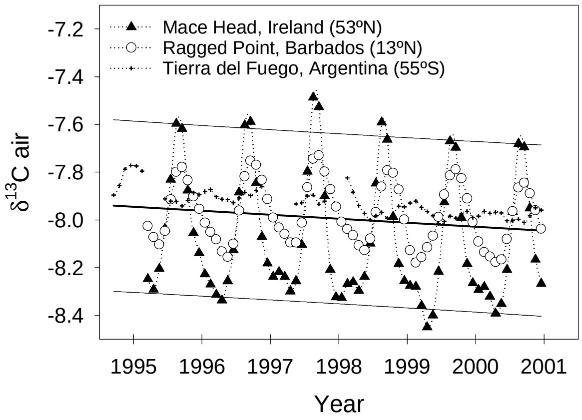


**Fig. II** Seasonal and interanual evolution of isotopic composition ($\delta^{13}C$) of atmospheric $CO_2$ in three locations, representative of Northern Hemisphere, Equatorial Belt and Southern Hemisphere (Mace Head, Ragged Point and Tierra del Fuego, respectively). For reference, the trend line calculated from all the points and its prediction confidence intervals are plotted. (data from CU-INSTAAR and NOAA-CMDL, ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/readme.html). Modified from Mateo *et al*. (2004).

time (at least over 4-5 years) or space (specially latitude). The vicinity (at the regional or local scale) of important sources of fossil fuel contamination should be also considered. For example, whereas in 1996 the average $\delta^{13}C$ in the Malta island was –8.0‰, the same value for Hungary was –8.3‰ (according to CU-INSTAAR and NOAA-CMDL, ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/readme.html).

### *II.2.1 Physiological basis of carbon isotope studies in plants*

The two stable carbon isotopes ($^{13}C$ and $^{12}C$) occur in the molar ratio of 1:99 in the atmosphere. However, plants with $C_3$ photosynthetic pathway generally contain proportionally less $^{13}C$ than does the air (Farquhar *et al.* 1989). Indeed, present $\delta^{13}C$ in air is about -8‰, whereas typical $C_3$ leaf composition becomes -29‰. In many plant physiological studies, carbon isotope discrimination ($\Delta^{13}C$) is calculated as follows (Farquhar *et al.* 1989):

**Eq. III**

$$\Delta^{13}\mathrm{C}_{\mathrm{plant}} = \frac{\delta^{13}C_{\mathrm{air}} - \delta^{13}C_{\mathrm{plant}}}{(1+\frac{\delta^{13}C_{\mathrm{plant}}}{1000})}$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{plant}$ refer to air and plant composition, respectively. This parameter reflects the amount in which the heavier isotope $^{13}C$ is discriminated respect the lighter $^{12}C$ during the physical and chemical processes involved in the synthesis of plant organic matter (Farquhar *et al.* 1989). The main factors determining $\Delta^{13}C$ in $C_3$ plants are diffusion in the air (including the boundary layer and the stomata) and carbon fixation by the carboxylating enzyme ribulose bisphosphate carboxylase (RuBisCO). Among the various models developed to describe isotopic discrimination in photosynthesis, the most extensively used is that of Farquhar *et al.* (1982):

**Eq. IV**

$$\Delta^{13}C\left(‰\right) = a\frac{C_a - C_\mathrm{i}}{C_a} + b\frac{C_i}{C_a} = a + \left(b - a\right)\frac{C_i}{C_a}$$

where $C_a$ and $C_i$ stand for ambient and intercellular partial pressures of $CO_2$, respectively, $a$ is the discrimination due to diffusion in air and $b$ is the discrimination due to carboxylation. Therefore, $\Delta^{13}C$ values in $C_3$ plants depend on the relative contribution of these two steps, determined by the relationship between stomatal conductance and photosynthesis. When stomata are open (fig. IIIa), $CO_2$ diffuses easily into the intercellular space, and $C_i$ is closer to $C_a$; thus, $\Delta^{13}C$ approaches the value of $b$ (about 30‰). In other words, RuBisCO is not limited by $CO_2$ and thus discrimination takes place mostly during the carboxylation step. In contrast, when stomatal conductance is reduced (fig. IIIb), $CO_2$ flux is limited and $C_i$ is significantly lower than $C_a$. Therefore, photosynthesis is strongly limited by stomatal conductance, and $\Delta^{13}C$ becomes closer to $a$, the value of the discrimination during $CO_2$ diffusion in air, (about 4.4‰).

### *II.2.2 Environmental conditions reflected in $\delta^{13}C$*

Following the aforementioned considerations, there are several environmental factors that can modify the isotopic composition of plant tissues through their influence on either leaf conductance or photosynthetic rate, or both parameters simultaneously. Changes in irradiance levels, $CO_2$ concentration, and plant water status (often derived from human activities) are clearly reflected in $\delta^{13}C$ variations. However, first systematic studies about plant $\delta^{13}C$ variability were focused on the identification of the recently discovered $C_4$ and CAM photosynthetic pathways (Ehleringer and Vogel 1993; Troughton 1979). Indeed, due to the strong correlation between photosynthetic rate and stomatal conductance, it was first considered that $C_i$ of plants would be constant, depending only on enzymatic and/or anatomical differences between different kinds of metabolism (Troughton 1979). Later, it was recognised that there is considerable variation in $C_i$ due to environmental factors, thus reflected in $\delta^{13}C$ of plants. Since then, much effort has been taken to understand the relationship between isotopic composition and environmental variation.

### Light intensity

Light intensity has been suggested to have a positive relationship with $\delta^{13}C$ as, in many studies, $\delta^{13}C$ of leaf tissue mimics irradiance gradients. This is the case, for example, when the vertical profile of $\delta^{13}C$ within a forest canopy is measured (Berry *et al.* 1997; Francey *et al.* 1985; Vogel 1978). However, interpretation of these results has been controversial as it is often difficult to discriminate direct light effects from those caused by differences in either $CO_2$ concentration, vapour pressure or air $\delta^{13}C$ throughout the canopy. Vogel (1978) attributed this "canopy effect" to a recycling of soil $CO_2$, which had lower (about 12‰) $\delta^{13}C$ than atmospheric $CO_2$. However, in those studies where simultaneous

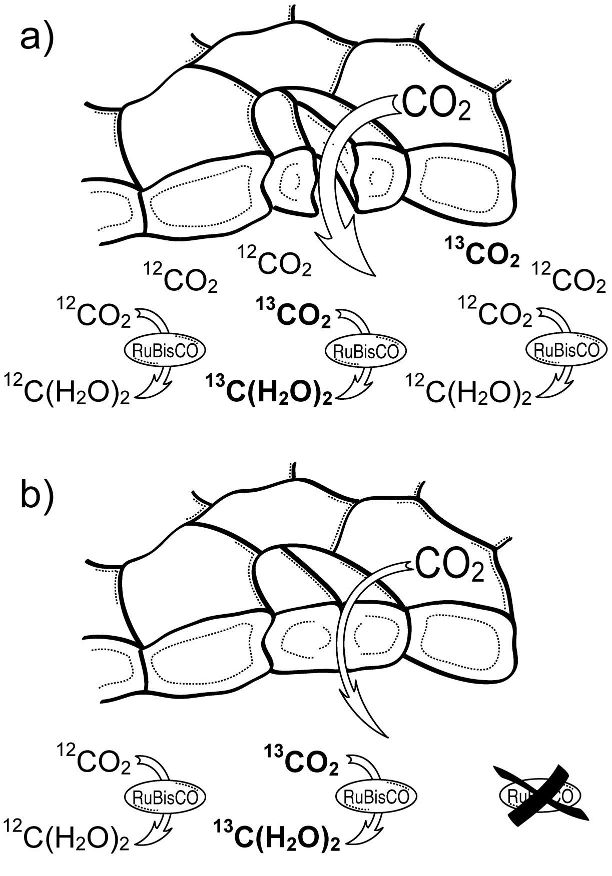


**Fig. III** Simplified scheme of the relationship between carbon isotope discrimination ($\Delta^{13}C$) in $C_3$ plants and stomatal conductance. A) High stomatal conductance, high discrimination: $CO_2$ diffusses easily into the intercelular space, the activity of the carboxylating enzyme (RuBisCO) is not limited by $CO_2$ concentration and thus it has more chances to discriminate against $^{13}C$. B) Low stomatal conductance, low discrimination: the flux of $CO_2$ is reduced, and the limiting factor of photosynthesis is stomatal conductance. In this case RuBisCO is forced to fix a higher proportion of $^{13}C$. Redrawn from Ferrio *et al.* (2003).

measurement of air $\delta^{13}C$ were performed (Berry *et al.* 1997; Francey *et al.* 1985), plant $\delta^{13}C$ was significantly correlated with sampling height, without a corresponding change in air $\delta^{13}C$. Therefore, most of the decrease in leaf $\delta^{13}C$ with canopy depth is likely to be related with stomatal and photosynthetic effects. This does not fully discard $CO_2$ and vapour pressure gradients as potential causes for the observed $\delta^{13}C$ variability within canopies (Broadmeadow *et al.* 1993).

Other works have shown strong evidence of a direct effect of light on $\delta^{13}C$, at least when this factor is limiting (Broadmeadow *et al.* 1993; Yakir and Israeli 1995; Zimmerman and Ehleringer 1990). Taking advantage of the negative relationship between leaf oxygen isotope composition ($\delta^{18}O$) and transpiration rates, (Yakir and Israeli 1995) demonstrated that the cause underlying the relationship between $\delta^{13}C$ of banana leaves and irradiance was an increase in photosynthetic capacity, without appreciable variations in stomatal conductance. Other authors arrived to similar conclusions combining gas-exchange measurements with $\delta^{13}C$ analyses (Broadmeadow *et al.* 1993). In contrast, Zimmerman and Ehleringer (1990) sustained that the origin of $\delta^{13}C$ increase in orchid leaves at high irradiance was due to declining stomatal conductance. This conclusion should be taken carefully, as it was only based on the small differences found in leaf nitrogen content, assuming that this would imply little change in potential photosynthetic capacity (Lambers *et al.* 1998).

### Atmospheric $CO_2$ concentration

Early in the 60s, Park and Epstein (1960) grew tomato plants at two levels of $CO_2$ and showed that plants grown at the higher level had a more negative $\delta^{13}C$ than plants grown at the lower concentration. Since then, similar results have been reported for several $C_3$ herbs (Beerling and Woodward 1995; Polley *et al.* 1993) and trees (Beerling 1997; Picon *et al.* 1997) over a wide range of $CO_2$ concentrations. In $C_3$ plants, $CO_2$ is usually limiting photosynthesis and, thus, an increase in $CO_2$ results in greater photosynthetic rates. On the other hand, plants take advantage of the increased $CO_2$ availability to augment water use efficiency (i.e. the ratio between net assimilation and water transpired) by closing stomata. Experimental results indicate that this reduction in stomatal conductance does not limit photosynthesis, thus $\delta^{13}C$ values become more negative as $CO_2$ concentration increases (Beerling and Woodward 1995; Polley *et al.* 1993). Therefore $CO_2$ and the above-mentioned light gradients may have additive effects within closed canopies, both contributing to leaf $\delta^{13}C$ decrease with depth (Berry *et al.* 1997; Broadmeadow *et al.* 1993).

### Water availability and water demand

Plants typically react against a decrease in water availability through stomata closure and, although carboxylation rates may also decline under water shortage, leaf conductance is usually affected to a larger extent, originating a reduction in $C_i$ and a concomitant increase in $\delta^{13}C$ (Farquhar *et al.*, 1989) (Scheidegger *et al.* 2000). Many studies under growth-chamber and field conditions have shown that plants developed under water stress (stress induced by low soil water content) produced leaves with higher $\delta^{13}C$ (see references in Ferrio *et al.* 2003; Griffiths and Parry 2002; Mateo *et al.* 2004; Warren *et* *al.* 2001). On the other hand, it is leaf water availability the factor that ultimately influences $\delta^{13}C$ and this availability depends not only on the water input from the soil, but also on its physical structure as well as on the hydraulic resistance along the plant xylem (Masle and Farquhar 1988; Warren and Adams 2000). Moreover, the rate of evaporation from the leaf also determines stomatal responses that subsequently affect $\delta^{13}C$. Indeed, an increase in the leaf-to-air vapour pressure gradient (VPG, which is considered the driving force for transpiration) will also cause a reduction in $C_i$, leading to higher $\delta^{13}C$ values (Barbour and Farquhar 2000; Ehleringer 1990). In accordance with these assumptions, it should be expected to find significant relationships between $\delta^{13}C$ and environmental parameters related with water availability or VPG, such as precipitation, relative humidity or potential evapotranspiration. Stewart *et al.* (1995), for example, analysed $\delta^{13}C$ from 12 plant communities along a rainfall gradient, finding significant negative correlations between annual rainfall and community-averaged $\delta^{13}C$. Similar relationships with water availability have been reported over smaller scales, reflecting microenvironmental changes (Ehleringer and Cooper 1988; Peñuelas *et al.* 1999). On the other hand, the expected positive correlation between VPG and $\delta^{13}C$ along climate gradients has also been reported elsewhere (Sparks and Ehleringer 1997; Williams and Ehleringer 1996).

It is important to note here, however, that in most cases the relationship between $\delta^{13}C$ and plant water availability is not linear, showing a saturation trend as water availability increases (Araus *et al.* 1997a; Korol *et al.* 1999; Warren *et al.* 2001). Warren *et al.* (2001), for example, found that, over a global survey of $\delta^{13}C$ values on conifers, $\delta^{13}C$ reached an asymptotic value once there is no water deficit, namely when the ratio between precipitation and evapotranspirative demand equalled unity. The reason for that general trend is that the main factor relating $\delta^{13}C$ with water inputs is stomatal conductance, which is expected to show progressively smaller changes as plants become less stressed. Under such conditions, other factors, such as altitude (Hultine and Marshall 2000), irradiance (Leavitt and Long 1991; Warren *et al.* 2001) or even nitrogen availability (Lopes *et al.* 2004; Peñuelas *et al.* 1999) might have as large an effect on $\delta^{13}C$ as climatic variables affecting plant water status, such as precipitation or evapotranspirative demand.

### II.3 Oxygen isotopes in plant physiology

The interpretation of oxygen isotope composition ($\delta^{18}O$) variability in plants is not as direct as that of $\delta^{13}C$, as it involves a combination of abiotic and biotic fractionation processes. The study of oxygen isotopes has been traditionally linked to that of the other element of water, hydrogen. In 1932, Urey *et al.* (1932) discovered a heavier form of hydrogen: Deuterium ($^{2}H$). Using measures of water density, they found considerable variations among samples of different origins. Years after the discovery of Urey, Dansgaard (1964) began a systematic analysis of $^{18}O$ and $^{2}H$ in marine and continental waters, finding that the first were more enriched in the heavier isotopes than the latter. On the other hand, the relationship between $\delta^{18}O$ and $\delta^{2}H$ in the world's fresh waters follows a predictable linear relationship, referred to as the "meteoric water line" (Craig 1961).

### *II.3.1 Environmental conditions reflected in $\delta^{18}O$*

Craig's meteoric water line is a direct consequence of the fact that fresh waters of warm regions have more enriched (positive) values of hydrogen and oxygen isotopes, whereas cool regions are isotopically depleted for both elements. Such differences are mostly due to fractionation occurring during phase changes in the course of the hydrological cycle (see Fig. IV). On the one hand, light isotopes ($^{16}O$ and $^{1}H$) evaporate more rapidly than their heavier counterparts, and thus water vapour is isotopically depleted respect source water (e.g. ocean water). On the other, the opposite occurs during precipitation. In order for water to condense and precipitate from an air mass, the temperature must drop. As temperature decreases, the heavier isotopes are selectively precipitated through distillation. This phenomenon is referred to as the "rainout" effect (Dansgaard 1964), which describes the successive depletion as air mass travels from its source and loses heavier isotopes. This effect can be enhanced by orographic effects that may cause an abrupt cooling of an air mass as it rises. On the other hand, the amount of this fractionation varies according to temperature and, as a consequence, isotopic composition of precipitation is positively correlated with temperature.

The source of water for most terrestial plants is soil moisture, so part of the signal in water isotopes (H and O) of plants come from the isotopic signature of precipitation. However, there are several potential fractionations before the water isotopes become fixed in plant tissues (Fig. IV). The first occurs within the soil, as evaporation affects the original isotopic signal, so the residence time and deep of soil water is important (Buhay and Edwards 1995). On the other hand, the observed discrepancy between the $\delta^{18}O$ of molecular oxygen in atmosphere (of photosynthetic origin) and marine water lead to suspect about some

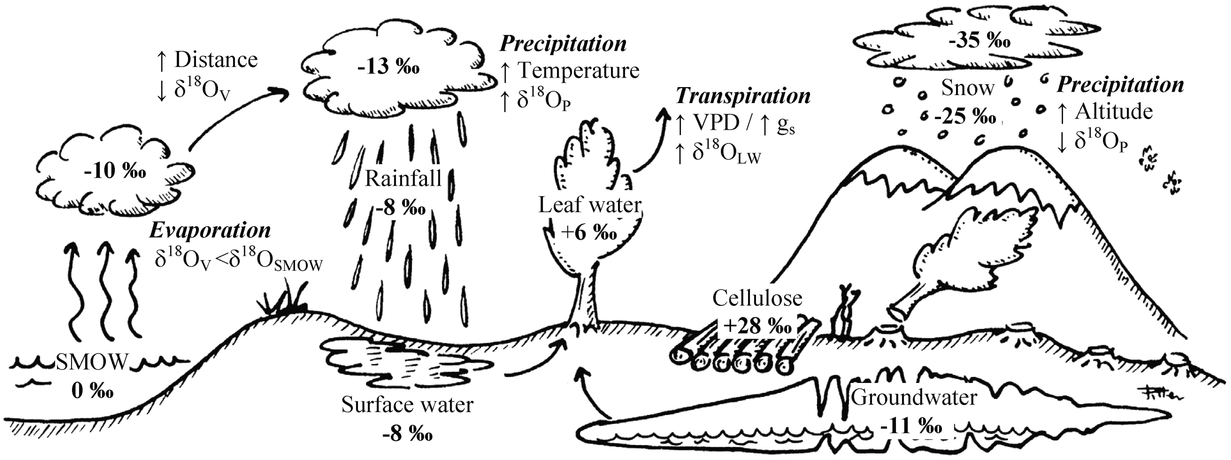


**Fig. IV** Main fractionation steps and typical values of oxygen isotope composition ($\delta^{18}O$) in a temperate climate. $\delta^{18}O_{SMOW}$, standard mean $\delta^{18}O$ in ocean water; $\delta^{18}O_V$, $\delta^{18}O_P$, $\delta^{18}O_{LW}$, $\delta^{18}O$ in water vapour, precipitation (either rainfall or snow) and leaf water, respectively; VPD, vapour pressure deficit; $g_s$, stomatal conductance. Original data from IAEA/WMO (2001) and Saurer *et al.* (1997b).

fractionation at the plant level. Gonfiantini *et al.* (1965) proved for the first time that the isotopic composition of leaf water was enriched in heavy isotopes during transpiration. When plant roots take soil water there is no fractionation, so the critical site of fractionation is the leaf. Summarising, the $\delta^{18}O$ of plant tissues reflects the variation in (1) $\delta^{18}O$ in source water, (2) evaporative enrichment of leaf water due to transpiration, and (3) biochemical fractionation during the synthesis of organic matter (Farquhar and Lloyd 1993; Yakir 1992). The level of enrichment of leaf water above source water ($\Delta^{18}O_e$) has been modelled as follows (Dongmann *et al.* 1974; Farquhar and Lloyd 1993):

**Eq. V**

$$\Delta^{18}O_e=\varepsilon^*+\varepsilon_k+(\Delta^{18}O_v-\varepsilon_k)\times(e_a/e_i)$$

where $\varepsilon^*$ is the proportional depression of vapour pressure by the heavier $H_2{}^{18}O$, $\varepsilon_k$ is the diffusion fractionation through stomata and leaf boundary layer, $e_a$ and $e_i$ stand for vapour pressure in atmosphere and intercellular space, respectively, and $\Delta^{18}O_v$ is the oxygen isotope composition of water vapour in the atmosphere (relative to source water). At constant temperature, and where source water and atmospheric vapour have the same isotopic signature, $\Delta^{18}O_e$ is linearly dependent on 1- $e_a/e_i$ (Barbour *et al.* 2001). According to this model, plants growing at higher humidity (i.e. higher $e_a$) are expected to have lower $\Delta^{18}O_e$. Consequently, within the same environmental conditions, plants with higher stomatal conductance (i.e. lower leaf temperature, which reduces $e_i$) are also expected to show smaller $\Delta^{18}O_e$. This models explains the isotopic enrichment of water at the site of evaporation, however, backward diffusion of the enrichment is opposed by the convection of isotopically lighter source water to the sites of evaporation, what is known as the *Péclet* effect (Barbour *et al.* 2004; Barbour and Farquhar 2000). The $\delta^{18}O$ of leaf water is, therefore, less depleted than that at the site of evaporation, so Eq. V overestimates the net effect of transpirative enrichment. Leaf water enrichment is passed on to the organic molecules formed in the leaf by exchange of oxygen atoms between carbonyl groups and water (Sternberg *et al.* 1986). The $\delta^{18}O$ of sucrose formed in the leaf is proportional to the signal of leaf water, although with 27‰ enrichment. However, in the case of stem cellulose, most of the enrichment signal (derived from sucrose) is further exchangeable with xylem water during the heterotrophic pathways of cellulose biosynthesis (Roden *et al.* 2000; Sternberg *et al.* 2003). This is particularly important for tree-ring studies, as will be discussed later (section III.1.7).

# III. Palaeenvironmental studies

Reconstruction of past climate is necessary to understand the context associated to some of the most intriguing natural processes: the evolution of species, their distribution or the occurrence of mass extinctions. The same is valid at the human sphere, as some crucial changes in the history of humanity (e.g. the onset of agriculture) cannot be dissociated from their environmental background. Moreover, a better understanding of past climate changes would be valuable to determine to what extent current global changes are unusual, as well as to improve long-term predictive models.

## III.1. How can we study past climates?

Due to the scarcity of instrumental climate records prior to the XXth century, estimates of global climate variability during past centuries must rely upon indirect “proxy” indicators. A proxy indicator is a local record (natural or documentary) that is interpreted using physical or biophysical principles to represent some combination of climate- or environmental-related variations back in time. Palaeoenvironmental proxies have the potential to provide evidence for large-scale climatic changes prior to the existence of instrumental or historical documentary records. Careful calibration and cross-validation procedures are necessary to establish a reliable relationship between a proxy indicator and the climatic variable or variables it is assumed to represent, providing a “transfer” function through which past climatic conditions can be estimated. High-resolution proxy climate indicators, including tree rings, corals, ice cores, and laminated lake/ocean sediments, can be used to provide detailed information on climate variations back in time, which can be complemented by certain coarser resolution proxy information (e.g. boreholes and non-laminated ocean sediment records). Some of the most commonly used proxy indicators are outlined below, paying special attention on tree-rings, due to their relative importance in the context of this Thesis.

### *III.1.1. Historical climatology*

Historical climatology is based on the study of different kinds of references that can be associated to climate events. For some areas, written evidences are available for several millennia and, although an explicit record of climate variables is not usual, they are often referred indirectly. Thus, references to the effects of flood events, long periods of drought or extremely cold winters can be found in religious archives in the form of rogation ceremonies, as well as in agricultural records (Barriendos and Martín-Vide 1998; Bell 1975; Pfister *et al.* 1999).

### *III.1.2. Ice-cores*

Ice cores from polar regions and mountain glaciers provide several indicators of past climates, including stable isotopes ($\delta^{18}O$), the fraction of melting ice, accumulation rates, concentration of different salts and acids, or the amounts of pollen or trace gases (e.g. $CH_4$ or $CO_2$) in the atmosphere, among others (Alley *et al.* 1997; Francey *et al.* 1999). Ice core data has a high temporal resolution (from annual to seasonal), and provides simultaneous information from climate-related and atmospheric variables, that might be helpful to assess the effect of $CO_2$ increase in global climate. The best dated series are based on sub-annual sampling of cores and the counting of seasonal ice layers, diving absolute errors of just a few years in a millennium (Folland *et al.* 2001). Ice cores have the disadvantage of being located far from the areas of human devolopment. This is a strength from the point of view of global climate issues, as they are less affected by local anthropogenic effects. However, it is also a disadvantage in order to assess the role of past climate changes in human evolution.

### *III.1.3. Pollen records*

The particular biochemical composition of pollen grains makes them relatively resistant to chemical, biological and physical damage. Consequently, pollen grains may survive millions of years in a large variety of sedimentary environments: peat bogs, lake and marine beds, several kinds of loose terrestrial sediments, and even in consolidated rocks (for example in stalagmites). As pollen deposition, for a given species, is expected to be proportional to its abundance, palynologists are able to reconstruct past vegetation from the study of fossil pollen assemblages. Furthermore, and provided a good knowledge of the ecological range of a given taxa, functional group or plant community, it has been possible to derive climatic information from pollen data (Lebreton *et al.* 2004; López-Sáez *et al.* 2003). Nevertheless, it should be noted that anthropogenic influence on vegetation has increased exponentially since the onset of agriculture. Thus, during great part of the Holocene, pollen data might have too human-derived noise to provide reliable climatic information, although still giving useful information about the landscape (Committee on abrupt climate change 2002; Lebreton *et al.* 2004). Indeed, most pollen assemblages for the last two millennia reveal clear symptoms of anthropogenic disturbance, such as the abundance of pollen from cereals and other crops, and the spread of ruderal species (Davis 1994; Lebreton *et al.* 2004; López-Sáez *et al.* 2003).

### *III.1.4. Lake and ocean sediments*

Annually laminated (varved) lake sediments also provide high-resolution records of palaeo-environment. When annual deposition of varves can be independently confirmed through other dating techniques (e.g. radiocarbon) they provide seasonal to inter-annual data. Three main climate variables may influence lake varves: summer temperature, winter snowfall and rainfall. Moreover to sedimentological studies (grain size, sedimentation patterns), lake sediments can be analysed from a variety of approaches: isotope analyses ($\delta^{13}C$ in organic matter, as a reflect of the dominant biota; $\delta^{18}O$ in carbonates and shells as indicator of evaporation rates), chemical analyses (changes in salt composition, abundance of different organic compounds, trace elements), pollen records, diatoms analyses (Davis 1994; Riera *et al.* 2004; Rodó *et al.* 2002).

Ocean sediments also offer high-resolution archives of climate, applying similar methods as those used in lake sediments. However, annually laminated sediments are not usual (Folland *et al.* 2001). Otherwise, sedimentation rates may still be enough to provide information on a century or millennial scale (Duplessy 2004; Moreno *et al.* 2004), based on radiocarbon or other external dating, such as volcanic ash shards. The range of variables that can be determined in marine beds is similar to that of continental lakes: sedimentology, stable isotopes in shells and organic matter, chemical analysis, pollen records (Committee on abrupt climate change 2002; Folland *et al.* 2001).

### *III.1.5. Coral reefs*

Annual growths of coral skeletons provide palaeoenvironmental information for tropical and subtropical oceans and atmosphere. For example, they have the potential to sample variations in regions sensitive to El Niño Southern Oscillation (ENSO) which can be useful to resolve large-scale patterns of climate (Folland *et al.* 2001). Accurate age estimates are possible for most sites using a combination of annual variations in skeletal density and geochemical parameters. Palaeoenvironmental reconstructions from corals rely mostly on geochemical variables, such as trace elements or stable isotopes ($\delta^{13}$C and $\delta^{18}$O) (Juillet-Leclerc and Yiou 2002; Rimbu *et al.* 2003).

### *III.1.6. Borehole measurements*

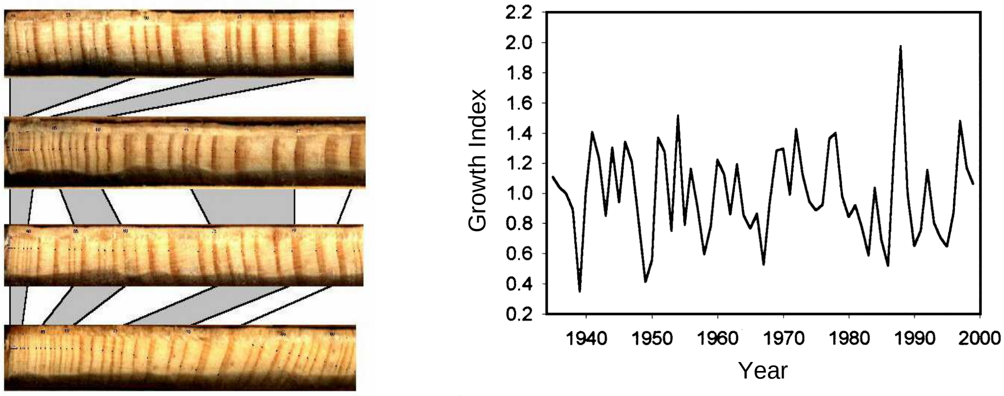
This is a relatively recent methodology, which attempts to provide a direct estimate of ground surface temperatures under certain simplifying assumptions about the geothermal properties of the earth. Although long-term temperature reconstructions have been made for the last two millennia (Bodri and Dovenyi 2004), they are based on assumptions not fully confirmed in such a large-scale, and the temporal resolution of borehole estimates decreases sharply back in time. Thus, borehole estimations of temperature are probably most useful for climate reconstructions over the last five centuries (Folland *et al.* 2001). Moreover, non-temperature related factors such as land-use changes make often difficult the interpretation of borehole data.

### *III.1.7. Tree-rings*

Since the early works of Douglass in 1914 (see (Robinson *et al.* 1990) for a historical review), tree-rings have been extensively used as palaeoenvironmental proxies. Currently, dendroclimatology is a well-developed subdiscipline of dendrochronology. It is based on the fact that tree growth is often limited by a variety of environmental factors, which may vary depending on the area studied (Cook and Kairiukstis 1990; Schweingruber 1988). For example, in arid and semiarid areas precipitation is the main factor limiting tree growth (Adams and Kolb 2004; Lev-Yadun *et al.* 1981; Serre 1976), whereas in colder regions temperature becomes the most limiting factor (Briffa *et al.* 2004; Panyushkina *et al.* 2003). As the objective is to maximise the effect of a given climate variable on tree growth, that is, to obtain a sensitive tree-ring series, the most useful trees are those found near the margins of their natural growth range (ecotones) where a clear limiting factor can be identificable. Indeed, over an altitudinal transect, for example, we may found significant differences in growth response (Adams and Kolb 2004; Gutierrez 1991; Tardif *et al.* 2003). As a rule, relatively isolated trees (i.e. without competition) but potentially limited by the desired factor, are the material of choice. For example, if we wish to obtain good inferences on precipitation, trees placed on slopes are preferable than those from the bottom of a valley.

## From individual tree-ring width series to large-scale chronologies

Tree growth is not only determined by climate, but is also affected by other, non-climatic factors. Tipically, ring width decreases exponentially with tree age, according to the geometry of an increasing trunk diameter (Kiviste *et al.* 2002; Rodriguez *et al.* 2003). However, competition and disturbance effects, among others, might alter this general trend. For example, growth rate of an individual tree can be greatly enhanced after a neighbour tree is cut. Thus, for environmental studies, this “undesired” variability should be statistically removed through standardisation. This involves fitting a curve to the tree-ring series, and then dividing each ring width value by the corresponding curve value. In an ideal tree, fitting an exponential curve would be enough, but in most cases a second detrending is performed through a cubic smoothing spline (Cook and Peters 1981). The spline acts as a high-pass filter, and is defined by a cut-off wavelength. For example, if this wavelength is 20 years, it will remove 50% of variance at this wavelength, with increasing percentages at longer wavelengths (97.5% at 50 years), and lesser removal at shorter wavelengths (2.5% at 8 years). In some cases, further detrending is performed through autoregressive models (Monserud 1986). The final result is always a series of indices with a long-term mean of unity, where higher or lower values for a given year represent proportionally higher or lower growths. To maximise the climate signal, the series obtained from several trees per site are averaged (preferably using a bi-weight robust mean estimation, (Cook and Holmes 1986) to build up a site chronology (Fig. V). The main handicap of statistical treatments is the risk of removing low-frequency



A) B)

**Fig. V** Example to illustrate the construction of a tree-ring chronology. Tree-ring cores of *Pinus halepensis* Mill., collected in a North-facing slope near Ribaroja d’Ebre (Ferrio and Voltas, unpublished results). A) visual cross-dating of cores from different trees; B) standardised chronology using a double detrending (exponential decay and spline smoothing with a cut-off wavelength of 25 years).

climate variability (Cook *et al.* 1995). In any case, the parameters of standardisation should be carefully determined, always bearing in mind the aim of our particular study.

To end, it should be noted that the time-span of tree-ring chronologies is not limited to the oldest living tree. Matching patterns in ring widths or other ring characteristics (such as ring density patterns) among several tree-ring series allow the identification of the exact year in which each tree ring was formed (cross-dating). This has allowed to construct long tree-ring chronologies, by overlapping extant ring width series with those obtained from old buildings, fallen logs, submerged trunks and other kinds of sub-fossil material. In Northern and Central Europe, pine and larch chronologies now extend more than 7000 years, and oak chronology extend over 9000 years, and attempts have been made to expand these up to 12000 years by linking the oak chronology with "floating" pine chronologies (Becker *et al.* 1991; Briffa and Matthews 2002; Friedrich *et al.* 2004; Pilcher *et al.* 1984).

### Stable isotopes in tree-rings

By the time it was first suggested the presence of a climatic signal in plant stable isotopes, tree-ring width analyses were being widely used in palaeoecological studies (Ehleringer and Rundel 1988; Robinson *et al.* 1990). Hence, the technique was rapidly implemented in tree-rings and several early studies on the relationship between stable isotopes and climate were performed on this material (Craig 1954; Epstein 1979; Gray and Thompson 1976; Libby *et al.* 1976). Since then, wood or cellulose isotopic composition of different elements has been extensively used in palaeoenvironmental and ecological studies (for further review see McCarroll and Loader 2004; Switsur and Waterhouse 1998; Warren *et al.* 2001).

Initially, variations in the $\delta^{13}$C of tree-rings was related to temperature (Stuiver and Braziunas 1987; Wilson and Grinsted 1977) and later studies have also reported positive relationships with this variable (Anderson *et al.* 1998; Saurer *et al.* 1995). However, according to current models of plant carbon discrimination (see section II.2), little or no direct effect of temperature on $\delta^{13}$C is expected. In fact, most of these studies found stronger relationships with other variables, such as relative humidity (Stuiver and Braziunas 1987) or precipitation (Anderson *et al.* 1998; Saurer *et al.* 1995). Hence, these results are probably derived from indirect relationships between temperature and plant water status. $\delta^{13}$C in tree-rings has shown to be strongly correlated to modelled soil water balance, precipitation and evaporative demand (Dupouey *et al.* 1993; Korol *et al.* 1999; Saurer *et al.* 1997c; Warren *et al.* 2001). However, such relationships appear to be restricted to seasonally dry climates: in other contexts, irradiance, altitude and nutrient availability appear to be the main responsive of isotopic variations (Hultine and Marshall 2000; Leavitt and Long 1991; Livingston *et al.* 1999; Warren *et al.* 2001).

Early studies on $\delta^{18}$O and $\delta^{2}$H in tree-rings found that they were strongly correlated with average temperatures, which was initially attributed to the isotopic fractionation in precipitation (Epstein 1979; Gray and Thompson 1976; Libby *et al.* 1976). However, more detailed physiological studies revealed that the variability associated to evaporative enrichment could not be neglected (Dongmann *et al.* 1974; Farquhar and Lloyd 1993). Thus, the observed correlations with temperature should be attributed to the synergistic effect of this variable on the isotopic signal in precipitation, and on evaporative enrichment (McCarroll and Loader 2004). On the other hand, evaporative enrichment also explains the strong effect of relative humidity on $\delta^{18}$O and $\delta^{2}$H reported by several authors (Burk and Stuiver 1981; Saurer *et al.* 1997a). Nowadays, $\delta^{18}$O and $\delta^{2}$H signatures in tree rings are known to be determined by three main variables (Roden *et al.* 2000; Sternberg *et al.* 2003): the isotopic signature of source water, leaf evaporative enrichment and exchange with xylem water during cellulose and/or lignin synthesis. The latter is determinant for the observed relationship between $\delta^{18}$O of tree-ring cellulose and meteoric water, as it enhances the source-water signal, softening the effect of leaf-level enrichment (Barbour *et al.* 2001; Saurer *et al.* 1997b; Sternberg *et al.* 2003).

Although most of the works cited so far were limited to the last 100 or 200 years, isotope studies in tree-ring have been also expanded over some large-scale tree-ring chronologies, providing high resolution climatic reconstructions throughout the Holocene (Becker *et al.* 1991; Feng and Epstein 1994; Kromer *et al.* 2004; Libby *et al.* 1976; McCornac *et al.* 1994).

### III.2. Holocene climate variability

After the cold-dry period known as *Younger Dryas* (ca. 12,800-11,500 cal. BP), begins the longest warm and stable period in the last 400,000 years, which defines the *Holocene* (Committee on abrupt climate change 2002; Folland *et al.* 2001). This period coincides with the epoch of greatest human development and, for some authors, was a crucial factor in the onset and spread of agriculture and modern civilisation. However, even being of lower magnitude than the preceding ones, a detailed study of Holocene climate reveals important climatic variations (Fig. VI). Between 11,500-10,500 cal. BP climate was still cooler than present, while from 9,000 to 8,200 cal. BP it became wetter and warmer than present. This was followed by a short cold period of about 200 years, around 8,200 cal. BP (Alley *et al.* 1997). Between 8,000 and 4,500 cal. BP climate was somewhat warmer and wetter than today, reaching since then environmental conditions relatively similar to those found in present times, except for some cold phases (900-300 BCE, Iron Age Cold Epoch; 1,600-1800 CE, Little Ice Age) (Gribbin and Lamb 1978; Van-Geel *et al.* 1998) and warm episodes (900-1400 CE, Medieval Warm Period) (Bradley *et al.* 2001; Gribbin and Lamb 1978).

Nevertheless, the implications of these global climate reconstructions can be very variable at the regional or local scale. In particular, precipitation regimes show a considerably spatial heterogeneity in response to global climate phenomenons, as is the case for the North Atlantic Oscillation (NAO) or the ENSO (Muñoz-Díaz and Rodrigo 2003; Rodó *et al.* 1997). The heterogeneous response of precipitation against global climate changes is mostly due to the multiplicity of factors that determine precipitation: circulation of air masses, orography, ocean temperature. This heterogeneity is not only evident from current meteorological data, but also from palaeoenvironmental registers. Thus, for example, the cold period occurring 8,000 years ago reveals a wetter/colder climate in NE Iberian Peninsula (Pérez-Obiol and Julià 1994), but it was drier in the South of Spain (Magny *et al.* 2003), as well as in the Near East (Van Zeist and Bottema 1988; Willcox 1999; Willcox 2002).

At the regional scale, climatic reconstructions of high temporal resolution are already available for the Iberian Peninsula. Most of these studies are based on the reconstruction of past vegetation, either from palinology (Jalut *et al.* 2000; López-Sáez *et al.* 2003; Pérez-Obiol and Julià 1994) or from charcoal studies (Allué 2002; Badal 1990). The study of vegetation, however, has the disadvantage of reflecting both climate and human effects, which reduces its palaeoclimatic significance after the onset of agriculture (Lebreton *et al.* 2004). Another important source of information is the reconstruction of lake levels, based on a combination of stratigraphic and geochemical methods (Riera *et al.* 2004; Rodó *et al.* 2002), which has provided useful data on temperature and water availability. These studies are complemented by the diverse techniques used to reconstruct marine temperature, in the Atlantic Ocean and in the Mediterranean Sea, as sea temperature has a strong influence on continental climate (see e.g. Moreno *et al.* 2004; Nebout *et al.* 2002). For the last millennium, these data has been complemented by other approaches, such as dendrochronology (Creus and Saz 1999; Manrique-Menéndez and Fernández-Cancio 2000; Richter and Eckstein 1990) or documentary studies (Barriendos 1997; Barriendos and Martín-Vide 1998).

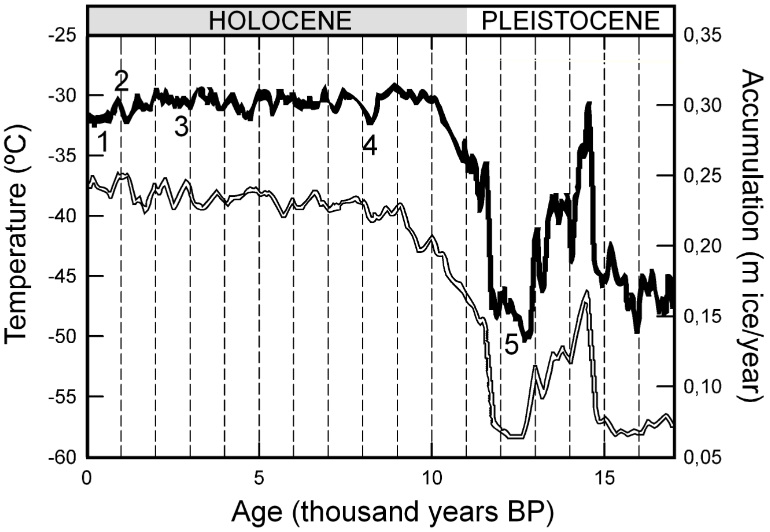


**Fig. VI** Climate changes in central Greenland over the last 17,000 years, at 100 years intervals. Reconstructions of temperature (black line) and snow accumulation rate (white line). Main climate events are highlighted: 1) Little Ice Age; 2) Medieval Warm Period; 3) Iron Age Cold Epoch; 4) 8k2 episode; 5) Younger Dryas. Adapted from Committee on abrupt climate change (2002).

# IV. Archaeobotany

In the process of excavation of archaeological sites one comes across plant matter of one kind or another. Most archaeological plant remains are found in a charred state and are often incomplete for standard systemathic studies. In the past, these botanical remains received very scant attention. Although some early studies date from the end of the XIXth century, the interest on plant remains did not began to generalise until the works of Helbaek (Helbaek and Schultze 1981) in the Near East. Since then, and due to its particular relevance for the origin of Old World agriculture, the Near East is still the region more extensively studied from archaeobotany (see e.g. Bar-Yosef and Kislev 1986; Hillman 1984; Renfrew 1973; Van Zeist and Casparie 1984; Willcox 1995). In the Iberian Peninsula, the earliest archaeobotanical works are those from Siret and Siret (1890), but the great impulse to the discipline came from Téllez and Ciferri (1954) and, in the 60’s and 70’s, from M. Hopf (Zohary and Hopf 1988). In the 80’s, the extensive work of Buxó (1997) helped to consolidate archaeobotanical studies in the area, that are now well developed around the Iberian Peninsula (Allué 2002; Alonso 1999; Peña-Chocarro 1992; Ros 1997).

## IV.1. Main approaches in archaeobotany

At the beginning, the interest of archaeobotany was centred on the origin of cultivated plants, from a genetic point of view, as well as on the processes associated to plant domestication and agriculture. Later, as the number of data available increased and new techniques became available, the initial interest on taxonomy *per se* derived into the study of the complex interactions between human communities and plants. This change in direction was evidenced through the foundation of the International Work Group of Palaeoethnobotany (IWGP) in 1968, which has served as a productive discussion forum for European archaeobotanists. Nowadays, the interest of archaeobotanists includes both the inference of plant exploitation strategies in the past and the reconstruction of palaeovegetation. For the latter, it is of special relevance a subdiscipline of archaeobotany, the anthracology or charcoal analysis, which is devoted to the study of wood remains. This approach has been well developed in Europe through the work of Vernet and his numerous co-workers and disciples (Bazile *et al.* 1977; Ros 1997; Vernet and Thiebault 1987). Nevertheless, the use of charcoal analysis to reconstruct vegetation is rather controversial, as it attempts to derive vegetation from material that has been collected by human groups, thus assuming that this would reflect plant communities. However, charcoal remains from archaeological contexts reflect the interaction between vegetation and their use by human groups. Some charcoal studies are indeed focused on the socio-economic (e.g. fuel exploitation, collection strategies) implications of this kind of remains (Piqué 1998; Willcox 1999). Thus, plant macroremains might provide also useful information from a palaeoenvironmental point of view, but the interpretation of results require a careful consideration of other factors determining the composition of plant assemblages.

## IV.2. Some methodological aspects

### *IV.2.1. Preservation of fossil plant remains*

In temperate climates, the most common way of preservation of plant remains is carbonisation. This is the case of wood charcoal, either derived from occasional fires or from the use of wood as fuel. Seeds and grains can also be burnt during different phases of food preparation (e.g. toasting), or included within animal dung used as fuel (Buxó 1997; Van Zeist and Casparie 1984). When plant materials are combusted under poor oxygen availability, they become carbonised, which prevents decomposition of plant remains by fungal or bacterial decay. However, as carbonisation increases, plant remains become more brittle, and thus the final preservation requires the ability to survive subsequent physical constraints during burial and recovery (Colledge 2001; Wright 2003). Plant remains can be also preserved in the form of mineralised tissues. In some cases, plant material may be preserved in an uncharred, dry, or saturated state. In very arid and desert climates, like ancient Egypt or coastal Peru, dried plant parts may last for millennia. Even in temperate climates, plants may be well preserved in dry caves and rockshelters. Saturated sites (bogs, swamps, lakes, etc) may also preserve fragile plant materials indefinitely, and this has been profited, for example, to build long tree-ring chronologies in Northern and Central Europe (McCarroll and Loader 2004).

### *IV.2.2. Recovering of plant remains*

Until relatively recently, most plant remains recovered from archaeological context were detected by chance. If a large quantity of charred seeds, or a big piece of wood, caught the eye of the archaeologist, this material would be gathered up and saved, otherwise not. However, small charred seeds and plant parts are not easily detected by the naked eye and, as a consequence, specialized means are necessary to obtain such remains. The most common method by which these smaller macrobotanical plant fragments are concentrated and recovered is through the technique known as flotation (see Fig. VII). Many different flotation systems have been devised, but all of them depend upon the same basic principle: if archaeological sediment is released into a container filled with water, then the sediment sinks and the charred plant remains float. There are several techniques of flotation (Buxó 1997): some techniques are multiple-operator, machine-assisted assembly lines, whereas others are simple, one-person bucket or barrel operations. It is sufficient to note here, however, that all of these systems are aimed at retrieving charred macrobotanical plant remains, and that the main purpose is the same for all: to concentrate and collect charcoal that is dispersed throughout archaeological deposits.

Although the use of flotation has found worldwide acceptance, not all archaeologists employ it as a technique to retrieve plant remains. In some areas, archaeological sediments may not be amenable to flotation. Even in cases where flotation is not physically possible, however, attempts are often made to recover plant remains. Dry or water sieving of sediments, for example, is routinely used in some European countries to obtain plant remains. On the other hand, the recovery and study of archaeobotanical remains is time-consuming and expensive and thus many archaeological projects simply do not have the necessary time and money. Fortunately, the interest on archaeobotanical studies is going beyond traditional archaeology, thus making accessible alternative sources for funding.

**Fig. VII** Examples of the main steps in the recovery of archaeobotanical remains. On the left, (top) partial view of the exacavated strata in Tell Halula, and (bottom) detail of charcoal remains included in the sediment. On the right, (top) extraction from the sediment of charcoal and charred seeds by flotation, and (bottom) archaeological grains of wheat and barley. Original pictures from J.L. Araus and R. Buxó.

### *IV.2.3. Dating of samples*

The dating of archaeobotanical remains is not a single issue. The radiocarbon dating method, based on the rate of decay of the unstable isotope $^{14}C$, is today the most widely applied dating technique for the late Pleistocene and Holocene periods (Taylor and Aitken 1997). $^{14}C$ is formed in the upper atmosphere through the effect of cosmic ray neutrons upon $^{14}N$. It is rapidly oxidised and transformed into $^{14}CO_2$, and subsequently fixed by plants. As soon as the plant dies, it ceases $^{14}CO_2$ uptake, and $^{14}C$ content in plant tissue decays progressively. In 1949, Libby, Anderson and Arnold were the first to measure the rate of this decay. They found that after 5568 years, half the $^{14}C$ in the original sample will have decayed and after another 5568 years, half of that remaining material will have decayed, and so on. The half-life is the name given to this value, which Libby measured at 5568±30 years. Currently, this value is considered to be 5730±40 years ("Cambridge" half-life). The initial solid carbon method developed by Libby and his collaborators was replaced with the Gas counting method in the 1950's. Liquid scintillation counting (LSC), utilising benzene, acetylene, ethanol, methanol etc, was developed at about the same time. Today the vast majority of radiocarbon laboratories use these two methods of radiocarbon dating. Their main handicap is the amount of sample required: even using small sample capabilities, about 100 mg are required for moderate precision datings. Of major recent interest is the development of the Accelerator Mass Spectrometry method of direct C14 isotope counting, which allows milligram sized samples to be dated. Radiocarbon dating should be calibrated externally to account for past changes in atmospheric $^{14}$C composition, and the final accuracy varies from several hundred years to a few decades, depending on the period considered (Jull 2005; Stuiver *et al.* 1998). On the other hand, the elevated cost of these analyses strongly limits the amount of samples to be dated. Consequently, most plant macroremains are assigned an age according to the dating obtained from other charcoal, seeds or bones from the same (or the nearest) stratigraphic unit. In some cases, a combination of stratigraphic and archaeological methods (e.g. based on ceramics and other artefacts) can provide more accurate dating than radiocarbon analyses. Dating of a given stratigraphic unit from wood charcoal has an additional uncertainty, as a given wood fragment might be older than the sediment in which is included. Thus, seeds and other short-living plant remains are often preferred for dating.

### IV.3. Stable isotopes in archaeobotanical remains

As discussed in sections II and III, stable isotopes in plant tissues reflect the environmental conditions in which they were developed. Charred archaeobotanical remains are indeed a subproduct of plant tissues, derived form their partial combustion, and thus may preserve some kind of environmental signal in their isotopic composition. De Niro and coworkers (DeNiro and Hastorf 1985; Marino and DeNiro 1987) were the first to propose the analyses of $\delta^{13}$C and $\delta^{15}$N in archaeobotanical remains, with the aim to provide reference values for dietary studies. They performed experimental carbonisations of several types of seeds, and assayed different treatments to remove soil contaminations, such as carbonates or humic acids, finding little change in $\delta^{13}$C due to carbonisation and chemical treatments, being greater for $\delta^{15}$N. Such an extensive work, along with the increasing physiological knowledge about $\delta^{13}$C variability in grain crops (Condon *et al.* 1987; Farquhar *et al.* 1982; Farquhar 1984), provided the necessary background for the first attempts to apply $\delta^{13}$C in archaeological grains to infer the water status of ancient crops (Araus *et al.* 1997a; Araus *et al.* 1999a; Araus and Buxó 1993). In these works, Araus and his colleagues developed quantitative models to estimate past water inputs in winter cereals (wheat and barley) through the analysis of $\delta^{13}$C in grains. Following a similar approach, and based on the close relationship between $\delta^{13}$C (as indicator of water status) and cereal yield (Araus *et al.* 1998; Condon *et al.* 1987; Voltas *et al.* 1998), they used $\delta^{13}$C of archaeological grains to infer ancient crop yields (Araus *et al.* 1999b; Araus *et al.* 2001; Araus *et al.* 2003).

From the point of view of climatic reconstruction, the analysis of crop remains has the disadvantage they may reflect either climatic or agronomic changes affecting crop water status (Araus *et al.* 1997b). However, provided an independent estimation of climate for the archaeological sites studied, this "handicap" might become an useful tool to reconstruct ancient agronomic practices. In this context, the use of charcoal remains of forest species appears to be the best alternative. The only studies available on the $\delta^{13}$C in ancient wood charcoal are those from February and Van der Merwe (1992) and Vernet *et al.*

(1996). Mostly based on the comparison with other palaeoenvironmental evidences, both concluded that past changes in the $\delta^{13}$C of charcoal could be related to changes in water availability, as occurred in intact wood (see section III.1.7). However, they did not studied the potential effect of carbonisation on wood $\delta^{13}$C, which, according to previous works, might be considerable (Florit 2001; Jones and Chaloner 1991). Consequently, prior to the application of stable isotopes in wood charcoal for palaeoenvironmetal studies, it is necessary to assess the effects of carbonisation under different conditions on the original environmental signal of wood.

# V. Objectives

As already stated in the overview, the general objective of this Thesis is to develop new tools in order to get insight into the climatic and agronomic conditions prevailing in ancient agriculture. Due to the interdisciplinary nature of this work, we have grouped the objectives into different topics:

## I. Reconstruction of climate

### Ecophysiological studies on extant trees

I.1 To compare the dependency of tree-ring growth and $\delta^{13}C$ on water availability for two Mediterranean species with different physiological strategies to cope with drought: the partially drought-tolerant *Quercus ilex* L., and the drought avoiding, *P. halepensis* Mill. We aim to assess our hypothesis that a drought-tolerant species, such as *Q. ilex* would show lower increase in Water Use Efficiency ($WUE_i$) under drier conditions than a drought-avoiding species (e.g. *P. halepensis*).

I.2 To determine which are the environmental variables (temperature, precipitation, vapour pressure deficit) determining $\delta^{18}O$ variation in whole wood and its main components (holocellulose and lignin) in a drought avoiding species, such as *P. halepensis*. We aim to test our hypothesis that, in a species with a strong stomatal regulation, the signal of source water ($\delta^{18}O_R$) would be lost due to the great variability in evaporative enrichment at the leaf level.

### Climatic inferences from modern wood

I.3 To determine to what extent different climatic variables (precipitation, temperature, evaporative demand) are reflected in wood $\delta^{13}C$ in *Q. ilex* and *P. halepensis*, in order to use $\delta^{13}C$ as a (palaeo)environmental proxy.

I.4 To assess the need for cellulose purification in *P. halepensis* in order to retrieve a climatic signal from $\delta^{13}C$ and $\delta^{18}O$ in wood. Cellulose purification is not possible in wood charcoal, so the lack of a climatic signal in lignin would preclude its use as a (palaeo)environmental proxy.

I.5 To assess, in *P. halepensis*, the potential complementarity of $\delta^{13}C$ and $\delta^{18}O$ analyses for (palaeo)environmental studies, as both are expected to respond in some way to stomatal processes.

### Stable isotopes in fossil charcoal and palaeoclimate

I.6 To assess the effect of carbonisation on the $\delta^{13}C$ of *P. halepensis* wood. In case of a significant effect, to develop a model to correct $\delta^{13}C$ shifts due to carbonisation.

I.7 To quantify changes in aridity in the Cinca and Segre Valleys (Ebro Depression) during the last four millennia, from the analysis of $\delta^{13}C$ in wood charcoal.

## II. Reconstruction of crop conditions

II.1 To update current models adressed to determine past water status in naked wheat (*Triticum aestivum/durum*) and hulled barley (*Hordeum vulgare*), and to apply them to ancient crops cultivated in NE Iberian Peninsula (from ca. 5,800 BCE to XIth century), SE Iberian Peninsula (*ca.* 4,500-300 BCE), as well as in the Fertile Crescent (*ca.* 7,900-6,500 BCE).

II.2 To explore new strategies to deal with $\delta^{13}C$ data on ancient crops, by comparing objectively the observed trends in different crop species, e.g. between cereals and legumes, or between wheat and barley. Specifically, we aim to find evidences that wheat was already favoured by ancient farmers, sowing it in better soils than barley, or under supplementary irrigation, and thus having better water status.

II.3 To develop a model to quantify original grain weight from the dimensions of charred cereal (wheat and barley) grains, to facilitate the comparison with modern agronomic data. Using this model, to assess the evolution of grain weight in cereals (wheat and barley) from the Cinca and Segre Valleys during the last four millennia. To find out potential reasons for the observed trends, by relating grain weight with estimations of crop water availability (through $\delta^{13}C$ of grains) and estimated precipitation (through $\delta^{13}C$ of charcoal).

II.4 By comparing observed trends in water availability for cereals in NW Mediterranean with those from fossil charcoal, to determine to what extent they can be attributed to climate or agronomic practices.

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# Chapter 1

*“Brotas derecha o torcida*
*con esa humildad que cede*
*sólo a la ley de la vida,*
*que es vivir como se puede.”*

Antonio Machado. *Las encinas*

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# $\Delta^{13}$C and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*

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**Abstract** Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill) are representative of two different functional types of trees extensively found in the Mediterranean: evergreen sclerophyllous and drought-adapted conifers. The former is considered a partially drought-tolerant species, whereas the latter is a typically drought-avoiding, water-saving species. We postulated that contrasting strategies in response to water deficits in *Q. ilex* and *P. halepensis* would lead to a differential sensitivity to changes in water availability. To test this hypothesis, we compared the response of both species in growth rate (measured as radial increments) and intrinsic water use efficiency ($WUE_i$, as inferred from carbon isotope discrimination -$\Delta^{13}$C- in wood samples) among sites from different provenance regions in NE Spain. We found significant differences in $\Delta^{13}$C and growth among provenance regions, partly explained by contrasting water availability. Wood $\Delta^{13}$C was positively related with precipitation and the ratio between precipitation and potential evapotranspiration (P/E). However, these relationships were stronger in *P. halepensis* (for P/E, $r^2$=0.67, p<0.001) than in *Q. ilex* ($r^2$=0.42, p<0.01). In addition, radial growth was positively related with precipitation and $\Delta^{13}$C in *P. halepensis* ($r^2$=0.32 and $r^2$=0.35, respectively, P<0.01), but not in *Q. ilex*. We concluded that *P. halepensis* was more sensitive than *Q. ilex* to water availability, showing faster increase in $WUE_i$ in response to water stress. We also found that the effect of North/South aspect on $\Delta^{13}$C and growth was site-specific, and unrelated to climatic variables.

**Keywords** climate • carbon isotope discrimination • water use efficiency • sclerophyllous • conifer

**Abbreviations** $\Delta^{13}$C, Carbon isotope discrimination; P/E, ratio between precipitation and potential evapotranspiration;WUEi, intrinsic water use efficiency

## 1.1. Introduction

Dominant tree species in Mediterranean ecosystems are either evergreen sclerophyllous or conifers adapted to the scarcity of water resources. Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill) are representative of each one of these functional types and both are extensively found in the Mediterranean. However, they show considerable differences not only on their structure but also on their ecophysiological responses to cope with drought. *Q. ilex* is a deep-rooted species that does not depend only on rainfall supplies but on a very effective water uptake (Valentini *et al.* 1992). It is also able to withdraw water from very dry soils, leading to the maintenance of relatively high stomatal conductances even during drought periods. Hence, it should be regarded as a partially drought-tolerant

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species (Damesin *et al.* 1998). In contrast, *P. halepensis* is known to reduce drastically water use during drought by stomatal closure (Borghetti *et al.* 1998). Its indeterminate free growth allows it to cease growth during drought events, recovering rapidly when water becomes available (Nicault *et al.* 2001; Liphschitz and Lev-Yadun 1986). Therefore, it can be considered as a true drought-avoiding species, fully adapted to effective water saving.

Carbon isotope discrimination ($\Delta^{13}C$) in $C_3$ plant tissues constitutes an integrated record of the ratio of intercellular to atmospheric concentration of $CO_2$ ($c_i/c_a$) during the period in which the carbon was fixed, and thus reflects the balance between assimilation rate and stomatal conductance (intrinsic water use efficiency, $WUE_i$). $\Delta^{13}C$ has been related to different variables associated with plant water stress, such as predawn water potential (Damesin *et al.* 1998; Warren *et al.* 2001), climatic gradients of humidity (Saurer and Siegenthaler 1989), precipitation (Korol *et al.* 1999; Schulze *et al.* 1998; Warren *et al.* 2001), and other indicators of water availability (Dupouey *et al.* 1993; Livingston *et al.* 1993). It may be advisable, however, to complement $\Delta^{13}C$ analyses with other indicators of plant function, such as morphological attributes (Damesin *et al.* 1997; Fleck *et al.* 1996) or plant growth (Dupouey *et al.* 1993; Brooks *et al.* 1998) to improve the understanding of the ecological meaning of $\Delta^{13}C$ values.

Information relating $\Delta^{13}C$ and water availability is still scarce for conifers from semiarid environments (Williams and Ehleringer 1996) and Mediterranean oaks (Damesin *et al.* 1998). At this point, we hypothesised that contrasting strategies in response to water deficits in *Q. ilex* and *P. halepensis* would lead to a differential sensitivity to changes in water availability, and that this could be tracked by means of $\Delta^{13}C$ analysis. To test this hypothesis, we compared the response of the two species in terms of radial growth rates and wood $\Delta^{13}C$ over a wide range of sites differing in precipitation regime. $\Delta^{13}C$ would provide an integrated record of $WUE_i$ during the growth period, whereas radial growth would indicate the effect of water shortage on the overall tree functioning. As a secondary objective, we studied the potential effect on $\Delta^{13}C$ and growth of increased evapotranspiration in South-faced slopes by sampling in opposite slopes (North/South).

## 1.2. Materials and Methods

### 1.2.1. Plant material

We sampled wood cores of Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) from different sites in NE Spain. As sampling strategy we used the current division in provenance regions of the distribution area for both species in Spain (Martín *et al.* 1998). A provenance region defines an adaptive unit in which phenotypically or genetically similar stands or seeds are found (European Council directive 1999/105/EC). A total of six provenance regions for *P. halepensis* and

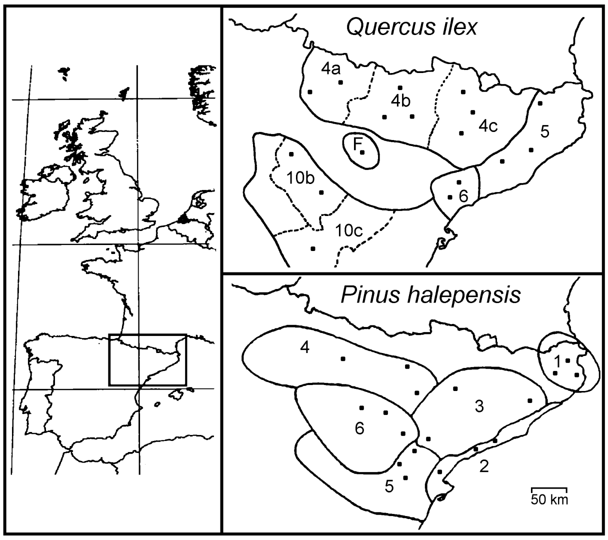

**Fig. 1.1** Geographical distribution of provenance regions of *Quercus ilex* and *Pinus halepensis* included in this work (see Table 1.1 for a detailed region description). Sampling sites within each region are marked as points. Adapted from Martín *et al.* (1998).

five provenance regions for *Q. ilex* were sampled (Fig. 1.1, Table 1.1). Due to their relative inhomogeneity, regions 4 and 10 for *Q. ilex* are divided into sub-regions (Martín *et al.* 1998), which will be taken here as separate regions. We selected sampling sites with available meteorological records within a 10km radius and representative of the environmental variability within each provenance region (one to three sites per region). Wood cores were extracted from the south side of mature, dominant and healthy trees, sampling four to six trees per aspect (North or South) within each site. *P. halepensis* cores were 5 mm in diameter, whereas for *Q. ilex* we took 12 mm-cores to allow for tree-ring dating, as described by Gené *et al.* (1993).

### 1.2.2. Tree-ring dating and sample preparation

Samples were oven-dried at 60ºC for 48h before being polished to allow tree-ring dating. We measured tree-ring widths with a binocular microscope coupled to a PC with the program TSAP v. 3.0 (Frank Rinn, Heidelberg). After visual cross-dating, assisted with tree-ring measurements, we selected a fragment of the cores corresponding with the periods 1975-1999 and 1980-2001, for *P. halepensis* and *Q. ilex*, respectively. We calculated mean annual radial growth from tree-ring widths within these periods as an indicator of long-term secondary growth. The fragments from the same site and aspect where then pooled and milled

**Table 1.1** Main climatic parameters for the provenance regions of *Quercus ilex* and *Pinus halepensis* included in this work. Inter-site range values shown between brackets.

| Region | P (mm) | T (ºC) | Ratio P/E | Im | Köppen classification |
|---|---|---|---|---|---|
| *Quercus ilex* L. | | | | | |
| 4a | 620 (561-678) | 14.1 (13.7-14.5) | 0.8 (0.8-0.9) | 0 | Cfa, Humid subtropical |
| 4b | 637 (449-1013) | 13.5 (12.9-14.2) | 0.9 (0.6-1.3) | 1 | Cfa, Humid subtropical |
| 4c | 616 (536-701) | 13.1 (11.6-14.3) | 0.8 (0.7-1.0) | -2 | Cfa, Humid subtropical |
| 5 | 851 (642-1019) | 12.8 (12.2-13.2) | 1.2 (0.9-1.4) | 30 | Cfb, Marine |
| 6 | 553 (529-576) | 13.8 (13.7-13.9) | 0.7 (0.7-0.8) | -7 | Csa, Pure mediterranean |
| 10b | 401 (392-410) | 11.6 (10.3-13.0) | 0.6 (0.6-0.7) | -20 | Csb, Coast mediterranean |
| 10c | 375 | 10.3 | 0.6 | -22 | BSk, Cool semiarid |
| F | 372 | 15.3 | 0.5 | -30 | BSk, Cool semiarid |
| Mean ± SD | 623 ± 221 | 13.3 ± 1.4 | 0.9 ± 0.3 | | |
| *Pinus halepensis* Mill. | | | | | |
| 1 | 744 (693-809) | 14.9 (14.1-15.4) | 1.0 (0.9-1.1) | 10 | Cfa, Humid subtropical |
| 2 | 493 (477-510) | 15.6 (15.2-15.9) | 0.6 (0.6-0.6) | -19 | Csa, Pure mediterranean |
| 3 | 566 (395-753) | 14.2 (12.7-15.1) | 0.7 (0.5-1.0) | -9 | Csa, Pure mediterranean |
| 4 | 497 (422-551) | 13.5 (12.9-14.0) | 0.7 (0.6-0.8) | -13 | Csa, Pure mediterranean |
| 5 | 450 (361-563) | 14.6 (13.2-15.9) | 0.6 (0.4-0.8) | -22 | Csa, Pure mediterranean |
| 6 | 385 (362-407) | 14.3 (13.5-14.9) | 0.5 (0.5-0.6) | -27 | BSk, Cool semiarid |
| Mean ± SD | 523 ± 140 | 14.4 ± 1.1 | 0.7 ± 0.2 | | |

P, annual precipitation; T, mean temperature; P/E, ratio between precipitation and potential evaporation; Im, Thornthwaite moisture index (Thornthwaite 1948; Köppen and Geiger 1936).

(IKA-A10) to a fine powder. We used intact wood tissue for isotopic analyses, without any chemical pre-treatment. The postulated need for cellulose purification from wood samples to assess environmental effects on $\Delta^{13}C$ has been questioned by many authors. Indeed, similar relationships can be found using either whole wood or cellulose (Warren *et al.* 2001; Korol *et al.* 1999).

### 1.2.3. Meteorological data

Meteorological data for both periods (1975-1999 for *P. halepensis* and 1980-2001 for *Q. ilex*) were supplied by the Instituto Nacional de Meteorología and the Confederación Hidrográfica del Ebro. We took monthly values for precipitation and mean temperature. From the temperature and the latitude, we calculated potential evapotranspiration following Thornthwaite (1948). The ratio between precipitation and potential evapotranspiration (P/E) was also calculated as an index of water availability. Wherever the altitude of the sampling site differed from that of the meteorological station, we applied the following correction (Gandullo 1994): for temperature, decrease in 0.6ºC per 100m; for precipitation, 8% increment precipitation is mostly convective, and not related with altitude.

### 1.2.4. Carbon isotope discrimination

The $^{13}C/^{12}C$ ratios of wood samples were determined by mass spectrometric analysis and results expressed as isotopic composition ($\delta^{13}C$). We calculated $\Delta^{13}C$ from $\delta_a$ and $\delta_p$ (air and plant $\delta^{13}C$), as defined by (Farquhar *et al.* 1982): $\Delta^{13}C = \delta_a-\delta_p/(1+\delta_p/1000)$. We took mean values of $\delta_a$=-7.7‰ and $\delta_a$=-7.8‰ for the periods 1975-1999 and 1980-2001, respectively, obtained from Francey *et al.* (1999) and the CU-INSTAAR/NOAA-CMDL network(ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/readme.html).

### 1.2.5. Statistical analysis

The data were subjected to analysis of variance (ANOVA), with species, provenance region, site nested to provenance region, and aspect included as factors in the model. Covariance analyses were performed to assess the influence of environmental variables on the aforementioned factors. Heterogeneity of slopes ANOVAs were performed to detect differences between species in the response of $\Delta^{13}C$ and radial growth to environmental variables (i.e. to test whether or not the regression coefficients are constant over groups, as assumed in a covariance analysis; Little *et al.* 1991). Simple correlations and linear regressions were calculated to assess relationships between variables. Unless otherwise stated, differences were considered statistically significant when $P < 0.05$.

## 1.3. Results

### *1.3.1. $\Delta^{13}$C and growth for Quercus ilex and Pinus halepensis*

$\Delta^{13}$C in *Q. ilex* varied among sites from 16.8‰ to 18.5‰, with a mean of 17.9‰. $\Delta^{13}$C was generally lower for *P. halepensis* (mean of 16.5‰) and showed greater range of variation (15.4‰-17.6‰). Differences in $\Delta^{13}$C between species were significant, even after removing the effect of climatic variables (precipitation, temperature, P/E) in a covariance analysis.

We also found significant differences in mean annual radial growth between species. They ranged from 0.3 to 1.1 mm year$^{-1}$ for *Q. ilex* (mean= 0.7 mm year$^{-1}$), whereas *P. halepensis* displayed greater values, ranging from 0.9 to 3.9 mm year$^{-1}$, with a mean of 1.9 mm year$^{-1}$.

### *1.3.2.Geographic variation*

There were significant differences in $\Delta^{13}$C among provenance regions for both species. For *Q. ilex* we found the highest and lowest values for $\Delta^{13}$C in regions 5 (18.5‰), and F (16.8‰), respectively (Fig. 1.2). For *P. halepensis*, mean values of $\Delta^{13}$C ranged from 15.9‰ in region 6 to 17.3‰ in the region 1. Differences between species were not detected for the North/South aspect. As for $\Delta^{13}$C, radial growth showed significant differences among provenance regions. We did not detect significant differences in radial growth due to North/South aspect, but there were significant interactions between aspect and provenance region for both species. Intra-site North-South differences ranged from –0.25 to +0.29 mm year$^{-1}$ for *Q. ilex*, and from –0.39 to +0.40 mm year$^{-1}$ for *P. halepensis*. However, these differences did not follow any clear pattern, being unrelated with either $\Delta^{13}$C or any climatic variable studied.

### *1.3.3. Relationship between climatic variables, $\Delta^{13}$C and growth*

#### Relationship with annual means

Site means of $\Delta^{13}$C were positively correlated with both annual precipitation and P/E for *Q. ilex* and *P. halepensis*, although these relationships were stronger for *P. halepensis* (Table 1.2). We also found a negative association between $\Delta^{13}$C and potential evapotranspiration for *P. halepensis* (Table 1.2). By plotting the relationship between precipitation (or P/E) and $\Delta^{13}$C, a trend towards

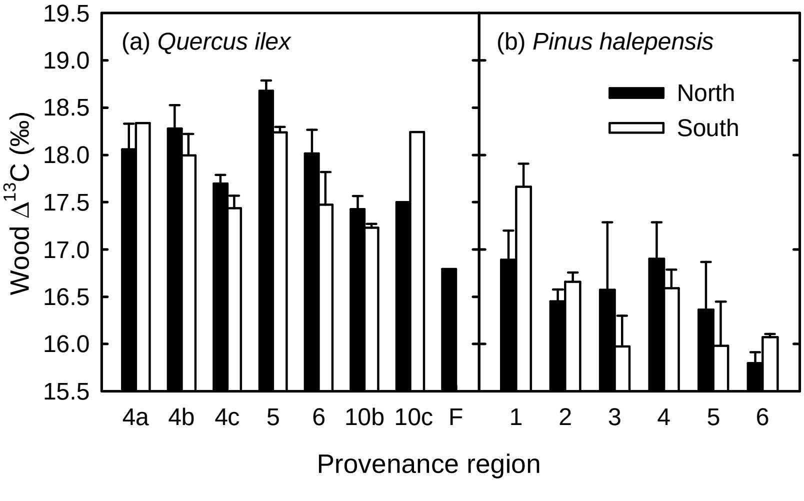

**Fig. 1.2** Mean wood $\Delta^{13}$C in *Q. ilex* and *P. halepensis* trees grown in two slope aspects (North/South) within the provenance regions described in Table 1.1. Error bars indicate the standard error of the means (N=1-3).

**Table 1.2** Across-sites correlation coefficients between annual means of climatic variables and either carbon isotope discrimination ($\Delta^{13}C$, ‰) or annual radial growth (mm) of *Quercus ilex* and *Pinus halepensis*.

| **Climatic variables** | ***Quercus ilex* (N=17)** | | ***Pinus halepensis* (N=18)** | |
|---|---|---|---|---|
| | **Wood $\Delta^{13}C$** | **Radial growth** | **Wood $\Delta^{13}C$** | **Radial growth** |
| Precipitation (P) | 0.59** | -0.11 | 0.73*** | 0.57*** |
| Mean temperature | -0.17 | -0.24 | -0.30 | 0.27 |
| Potential evapotranspiration (E) | -0.30 | -0.28 | -0.46* | 0.13 |
| Ratio P/E | 0.63*** | -0.08 | 0.77*** | 0.53*** |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

saturation was observed as water availability increased (i.e. precipitation and P/E) (Fig. 1.3). Thus, a log transformation fitted better the data. *Q. ilex* $\Delta^{13}C$ increased with the log of precipitation at a rate of 0.97‰ per log unit, with a coefficient of determination ($r^2$) of 0.36 (Fig. 1.3a), whereas the increase for *P. halepensis* was 1.91‰ per log unit ($r^2=0.59$). A log transformation for P/E gave similar fits, with $r^2=0.42$ and $r^2=0.67$ for *Q. ilex* and *P. halepensis*, respectively (Fig. 1.3b). We detected strongly significant differences ($P<0.001$) between species when comparing the slopes of the relationship between log transformed data (either precipitation or P/E) and $\Delta^{13}C$.

Site mean annual radial growth was not associated with climatic variables for *Q. ilex*, whereas it was positively related with precipitation and P/E for *P. halepensis* (Table 1.2). Radial growth of *P. halepensis* increased linearly with annual precipitation at a lapse rate of 3.31 μm year$^{-1}$ mm$^{-1}$ (Fig. 1.4a). Radial growth was also positively related with $\Delta^{13}C$ in *P. halepensis* (Fig. 1.4b), but not in *Q. ilex*.

### Relationship with monthly means

Correlations between *Q. ilex* $\Delta^{13}C$ and monthly precipitation were generally higher in autumn-winter than in spring-summer months, of which

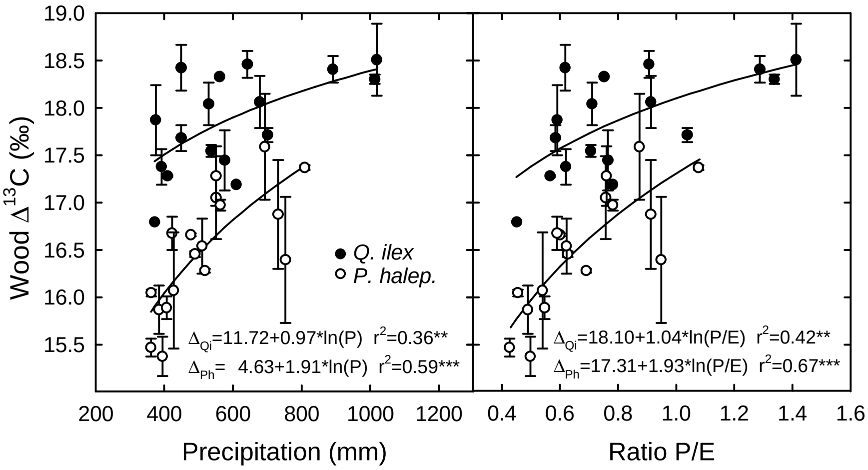

**Fig. 1.3** Relationship between wood $\Delta^{13}C$ in *Q. ilex* and *P. halepensis* and either mean annual precipitation (P) or the ratio between precipitation and potential evapotranspiration (P/E). $\Delta_{Qi}$ and $\Delta_{Ph}$ represent $\Delta^{13}C$ in *Q. ilex* and *P. halepensis*, respectively. Error bars indicate the standard error of the means (N=2). * $P<0.05$; ** $P<0.01$; *** $P<0.001$

only June showed a significant relationship (Fig. 1.5a). Conversely, *Q. ilex* $\Delta^{13}C$ was not significantly correlated with monthly values of potential evapotranspiration, although some negative trend was observed for summer months (Fig. 1.5b). $\Delta^{13}C$ of *P. halepensis* showed significant correlations with monthly precipitation throughout the year (Fig. 1.5c). We also found a significant, negative relationship between *P. halepensis* $\Delta^{13}C$ and potential evapotranspiration, but only during summer (from June to September, see Fig. 1.5d).

There was a significant, negative relationship between radial growth and September precipitation for *Q. ilex* (Fig. 1.5a), whereas correlations between radial growth and potential evapotranspiration, although slightly negative throughout the summer months, were still not significant (Fig. 1.5b). Radial growth of *P. halepensis* was significantly correlated with monthly precipitation in March and from July to December (Fig. 1.5c), being positively associated with potential evapotranspiration in winter (December-February, Fig. 1.5d).

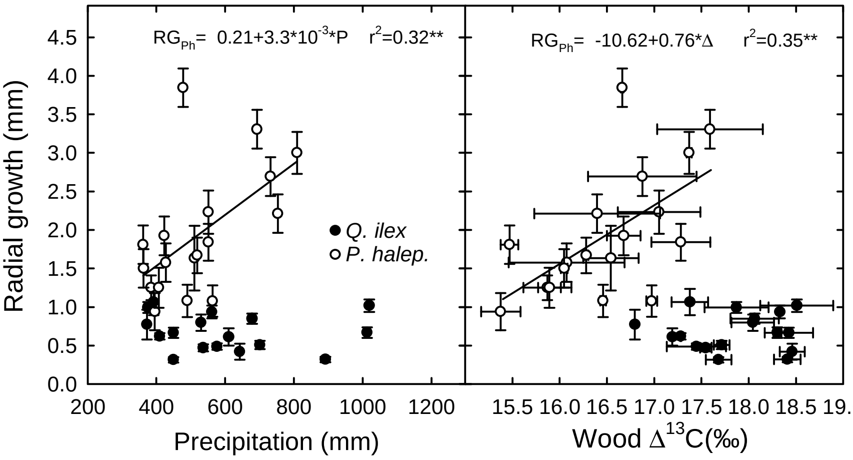

**Fig. 1.4** Relationship between mean radial growth (RG) in *Q. ilex* and *P. halepensis* and either mean annual precipitation (P) or wood $\Delta^{13}C$ ($\Delta$). RG$_{Ph}$, radial growth in *P. halepensis*. Error bars indicate the standard error of the means (N=8-12). * $P<0.05$; ** $P<0.01$; *** $P<0.001$

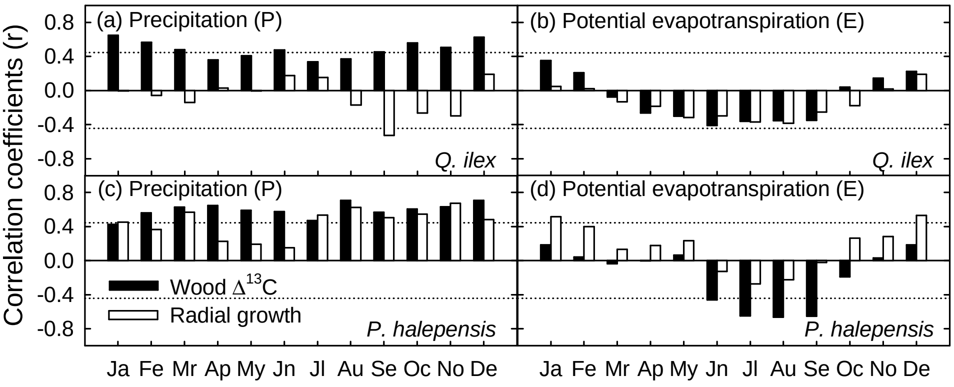

**Fig. 1.5** Correlation coefficients between wood $\Delta^{13}C$ / radial growth and either monthly precipitation (a, c) or monthly potential evapotranspiration (b, d) for *Q. ilex* (a, b) and *P. halepensis* and (c, d). Dotted lines indicate the threshold-value for significant correlations ($P<0.05$).

## 1.4. Discussion

### 1.4.1. $\Delta^{13}C$ response to water availability and climate

Wood $\Delta^{13}C$ in *Q. ilex* and *P. halepensis* was positively related with annual precipitation and the ratio P/E, thus reflecting the effect of both climatic variables on plant water status. Several authors have reported similar relationships between indicators of water availability and wood $\Delta^{13}C$, either in conifers (Korol *et al.* 1999; Warren *et al.* 2001) or in broadleaf trees (Saurer *et al.* 1995; Dupouey *et al.* 1993). Previous works in conifers have shown that $\Delta^{13}C$ is more sensitive to water availability as water becomes more scarce, reaching an asymptotic level around P/E>1 (Korol *et al.* 1999; Warren *et al.* 2001). Although our climatic range exceeds only slightly this threshold, models greatly improved after log transformation of the variables. Our results thus confirm that P/E exerts an important control on $\Delta^{13}C$, but only when water availability is the main limiting factor for photosynthesis.

On the other hand, $\Delta^{13}C$ was not significantly related with temperature, although previous studies placed temperature among the factors best correlated with $\Delta^{13}C$ (Stuiver and Braziunas 1987; Anderson *et al.* 1998; Brooks *et al.* 1998). The relatively narrow range of temperatures (10.3-14.5ºC for *Q. ilex* and 12.7-15.9ºC for *P. halepensis*), as well as the absence of cold climates in this range, might explain the lack of temperature effect on $\Delta^{13}C$. Nevertheless, a mechanistic explanation for a negative relationship between temperature and $\Delta^{13}C$ still lacks, and often it can be attributed to indirect correlations with other variables, such as relative humidity (Stuiver and Braziunas 1987), precipitation (Anderson *et al.* 1998) or potential evapotranspiration (Brooks *et al.* 1998).

Differences in $\Delta^{13}C$ due to the slope aspect were generally low and dependent on the study site. This suggests that the aspect effect on plant water status was not only exerted through the influence of an increased irradiance on evapotranspiration. Another variable that should be considered is wind regimes, which markedly influences leaf temperature and evapotranspiration rates (Jones 1992). This could be the case, for example, of *P. halepensis* in region 1, characterised by strong cold-dry winds (*tramuntana*) coming from the North which could increase water demand on North-faced slopes. Nevertheless, we can not discard the effect of additional intra-site heterogeneity due to soil substrate, tree age or tree density (the latter, for example, was often higher in North-facing slopes).

It should be noted that, when comparing samples from different geographic origins, differences in $\Delta^{13}C$ might be partly due to adaptive (i.e. genetic) variation in $WUE_i$ among populations (Schulze *et al.* 1998; Anderson *et al.* 1996). This can bring about an additional noise to the climatic signal present in $\Delta^{13}C$, which can be either enhanced or decreased (Schulze *et al.* 1998). In a provenance trial of *P. halepensis*, Tognetti *et al.* (1997) found lower stomatal reduction in seedlings of xeric origins. If this was the case in our study, genetic variation would have reduced the variability in $\Delta^{13}C$ among provenance regions. We are unaware of similar studies for *Q. ilex*, but genetic variability in water relations can be considerable, even within populations (Leiva and Fernández-Alés 1998). Nevertheless, environment is by far the main factor responsive for $\Delta^{13}C$ variation in $C_3$ plants. Indeed, the extreme regional $\Delta^{13}C$ values found in our study matched well the outermost values of precipitation and P/E within each species (see Table 1.1, Fig. 1.2). In addition, when we introduced P/E in a covariance model for $\Delta^{13}C$, this term explained about 45% and 67% of the variability due to provenance regions for *Q. ilex* and *P. halepensis*, respectively.

### 1.4.2. Can we track differences in drought response through $\Delta^{13}C$?

Although both species showed a close relationship between water availability and $\Delta^{13}$C, the regression slopes of $\Delta^{13}$C on either precipitation or P/E (on a log basis) were about two-fold greater for *P. halepensis* than for *Q. ilex*. Several works have reported faster stomatal closure under drought for *P. halepensis* than for *Q. ilex*. Whereas *P. halepensis* exhibits a rather high threshold of predawn water potential for stomatal closure (from -0.8 to -1.5 MPa; Borghetti *et al.* 1998), it is considerably lower for *Q. ilex* (from -3.0 MPa to -5.0 MPa; Damesin *et al.* 1998). This translates into lower $\Delta^{13}$C sensitivity to water availability in *Q. ilex* than in *P. halepensis*. Moreover, *Q. ilex* is a deep-rooted tree and thus can rely on groundwater during drought periods. Correlations between $\Delta^{13}$C and monthly precipitation for *Q. ilex* were about two-fold higher in winter than in summer (see Fig. 1.5), suggesting that this species relies mostly on groundwater during the drier months. In contrast, the relationship between $\Delta^{13}$C and precipitation was nearly constant throughout the year in *P. halepensis*, in concordance with the higher dependence on rainfall of Mediterranean conifers (Valentini *et al.* 1992; Nicault *et al.* 2001).

### 1.4.3. Radial growth response to water availability and climate

Since the same range of radial growth could be found for *Q. ilex* over the whole range of precipitation, we can conclude that long-term radial growth in populations from drier sites was not water-limited. Plants growing under dry environments may show several adaptations, either phenological or physiological, allowing them to keep growth rates similar as those shown in wetter sites. For example, *Q. ilex* shows greater allocation from leaves to xylem in sites with decreased water availability (Villar-Salvador *et al.* 1997). In this case, overall biomass could increase with water availability, without significant changes in radial growth rates. In addition, *Q. ilex* can rely on groundwater for growth, even under very dry climates (Valentini *et al.* 1992). Remarkably, the only significant (negative) relationship between radial growth in *Q. ilex* and precipitation was found for September. In fact, greater precipitation during early-autumn is often related with an increased seasonality in Mediterranean environments. *Q. ilex* would display higher radial growths in climates with lower seasonality, regardless of the total amounts of precipitation. This suggests that *Q. ilex* still preserves some physiological features from its arcto-boreal origin which are “out of phase” with the requirements of a summer-dry habitat (Liphschitz and Lev-Yadun 1986).

In contrast, radial growth in *P. halepensis* was highly dependent on water availability, as it was correlated with both precipitation and $\Delta^{13}$C. Moreover, a covariance analysis revealed that precipitation described about 65% of the variability in radial growth due to provenance regions. Previous work found a strong intra-site relationship between radial growth and precipitation in *P. halepensis* (Serre 1976). Our results show that this relationship also holds for inter-site variability. As compared to winter, the lack of a relationship between monthly precipitation and radial growth during spring has also been observed in other conifers (Lebourgeois 2000), since spring radial growth is partly supported by previous-year photosynthates (Lebourgeois 2000; Fallas 1970). *P. halepensis* is considered a very plastic species that shows two periods with reduced cambial activity: one is defined by the summer drought and the other by low winter temperatures (Liphschitz and Lev-Yadun 1986). In our study, the positive association between potential evapotranspiration and radial growth during the coldest months suggests that growth was also limited to some extent by winter cold (cf. Fig. 1.5).

### *1.4.4. Concluding remarks*

From our results, we can conclude that both *Q. ilex* and *P. halepensis* respond to a decrease in water availability by increasing their $WUE_i$, as inferred from wood $\Delta^{13}C$. However, *P. halepensis* was more sensitive to water availability, showing a steeper decrease in $\Delta^{13}C$ with increasing aridity. This is in agreement with the hypothesis that a partially drought-tolerant species, such as *Q. ilex*, would show lower increase in $WUE_i$ under drier conditions than a drought-avoiding species (e.g. *P. halepensis*). The fact that radial growth was related with precipitation and $\Delta^{13}C$ in *P. halepensis* but not in *Q. ilex* further confirms that *P. halepensis* is more dependent on precipitation in order to thrive and survive.

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# Chapter 2

*“És el vent, per altra part, que treu dels pins de la costa el rendiment més tangible; el gregal en treu un perfum sec i crepitant; el garbí, més humit, gairebé femení, embriagant.”*

Josep Pla. *El meu país*

J.P. FERRIO[1]* • J. VOLTAS[1]

# Carbon and oxygen isotope ratios in wood constituents of *Pinus halepensis* as indicators of precipitation, temperature and vapour pressure deficit

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**Abstract** Carbon and oxygen isotope compositions ($\delta^{13}C$, $\delta^{18}O$) in tree-rings have been shown to bear relevant climatic signals. However, little is known about the interrelation between both isotopes in wood constituents, and only referred to species from relatively wet climates. We hypothesized that, in a species adapted to temporary droughts (e.g. *Pinus halepensis* Mill.), the signal derived from $\delta^{18}O$ in precipitation would be hidden by the strong variability in leaf transpirative enrichment. To test this assumption, we compared the effect of precipitation, temperature and vapour pressure deficit (VPD) on $\delta^{18}O$ and $\delta^{13}C$ along 23 sites covering the ecological range for this species. We extracted the cores from the south side of 4-6 adult, dominant trees per aspect (north/south) within each site. For each aspect and site, fragments of the period 1975-1999 were pooled and milled to a fine powder. To further test the postulated need for cellulose purification in the assessment of climatic information, we studied these relationships in whole and extracted wood, holocellulose and lignin. In all wood fractions, d13C was related to annual precipitation (r=-0.58** to -0.78***) and VPD (r=0.53** to 0.57**). In contrast, for $\delta^{18}O$ only holocellulose showed consistent relationships with climatic data, being strongly significant for VPD (r=0.66***). However, it was unrelated to modelled $\delta^{18}O$ in precipitation, confirming that transpirative enrichment (driven by VPD) dampened the source signal in *P. halepensis*. The relationships between $\delta^{13}C$ and $\delta^{18}O$ were generally poor, regardless of the wood constituent, suggesting that, although both variables were somewhat related to transpirative demand, they were relatively independent. This was further confirmed by building stepwise models using both isotopes to predict annual and seasonal precipitation ($r^2$=0.34** to 0.68***), temperature ($r^2$=0.15* to 0.37**) and VPD ($r^2$=0.31** to 0.55***). We concluded that, even describing partially the same climate variables, the information underlying the two isotopes can be regarded as complementary.

## 2.1. Introduction

Carbon and Carbon and oxygen isotope ratios from tree-rings offer the possibility of high-resolution records of past climate. Although both isotopes have been related to similar environmental variables, the processes leading to such relationships differ substantially between them. Carbon isotope composition ($\delta^{13}C$) of plants is isotopically depleted in $^{13}C$ respect the atmospheric $CO_2$, which is the carbon source for photosynthesis. The instantaneous values of $\delta^{13}C$ in $C_3$ plants are determined by the ratio of intercellular to atmospheric partial pressure of $CO_2$ ($p_i/p_a$), reflecting the balance between diffusion in the intercellular space (mostly through stomata) and carbon fixation by the carboxylating enzyme.

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Consequently, $\delta^{13}C$ in dry matter constitutes an integrated record of the plant water status during growth (Farquhar *et al.* 1982). $\delta^{13}C$ in wood is therefore expected to integrate the effect of different environmental variables on the water status of trees. Indeed, $\delta^{13}C$ has been related to various climatic variables, such as humidity or temperature (Anderson *et al.* 1998; Hemming *et al.* 1998), and several indicators of water availability (Dupouey *et al.* 1993; Ferrio *et al.* 2003a; Hemming *et al.* 1998; Saurer *et al.* 1997a).

The association between oxygen isotopes and environmental variables, however, is less clear. The oxygen isotope composition ($\delta^{18}O$) of plant tissues reflects the variation in (1) $\delta^{18}O$ in source water, (2) evaporative enrichment of leaf water due to transpiration, and (3) biochemical fractionation during the synthesis of organic matter (Farquhar and Lloyd 1993; Yakir 1992). Generally, the $\delta^{18}O$ of source water is strongly dependent on that of rain water ($\delta^{18}O_R$). $\delta^{18}O_R$ is mainly determined by the temperature of droplet formation, being higher with higher temperatures (Dansgaard, 1964). Other factors, such as altitude or precipitation, also affect $\delta^{18}O_R$ (Barbour *et al.* 2001). The level of enrichment of leaf water above source water ($\Delta^{18}O_e$) has been modelled as follows (Dongmann *et al.* 1974; Farquhar and Lloyd, 1993):

**Eq. 2.1**

$$\Delta^{18}O_e=\varepsilon^*+\varepsilon_k+(\Delta^{18}O_v-\varepsilon_k)\times(e_a/e_i)$$

where $\varepsilon^*$ is the proportional depression of vapour pressure by the heavier $H_2{}^{18}O$, $\varepsilon_k$ is the diffusion fractionation through stomata and leaf boundary layer, $e_a$ and $e_i$ stand for vapour pressure in atmosphere and intercellular space, respectively, and $\Delta^{18}O_v$ is the oxygen isotope composition of water vapour in the atmosphere (relative to source water). According to this model, plants growing at higher humidity (i.e. higher ea) are expected to have lower $\Delta^{18}O_e$ (for further details, see Farquhar and Lloyd 1993). Within the same environmental conditions, plants with higher stomatal conductance (i.e. lower leaf temperature, which reduces ei) are also expected to show smaller $\Delta^{18}O_e$. Leaf water enrichment is passed on to the organic molecules formed in the leaf by exchange of oxygen atoms between carbonyl groups and water (Sternberg *et al.* 1986). However, in the case of cellulose, which is the main component of wood, about 50% of the enrichment signal (derived from sucrose) is further exchangeable with xylem water. Such exchange is determinant for the observed relationship between $\delta^{18}O$ of tree-ring cellulose and $\delta^{18}O_R$, as it enhances the source-water signal, softening the effect of leaf-level enrichment (Anderson *et al.* 1998; Barbour *et al.* 2001; Saurer *et al.* 1997b).

Traditionally, tree-ring analyses have been performed on purified cellulose. However, recent works have shown strong relationships between the isotopic composition ($\delta^{13}C$ or $\delta^{18}O$) of cellulose and other wood fractions, suggesting that this time-consuming process might be obviated (Barbour *et al.* 2001; Borella *et al.* 1998). On the other hand, although the association between either $\delta^{13}C$ or $\delta^{18}O$ and environmental variables has been widely studied, the information available about the interrelation between both isotopes in wood cellulose is still scarce (Anderson *et al.* 1998; Saurer *et al.* 1997a), and we are not aware of such studies for other wood fractions. Moreover, the results from these works, performed in temperate areas, are not necessarily extrapolable to Mediterranean species, as they are affected by different environmental constraints and may respond in a different way to the same climatic variables. This might be the case of the species chosen in this study, Aleppo pine (*Pinus halepensis* Mill), which is highly sensitive to changes in water availability as an adaptive strategy to the great inter-annual and seasonal variability of Mediterranean climates. Thus, it is able to reduce drastically growth and water expense during drought by stomatal closure, recovering rapidly when water becomes available (Borghetti *et al.* 1998; Ferrio *et al.* 2003a). We hypothesized that, in a species with such responsive stomata, the potential $\delta^{18}O_R$ signal in wood tissues would be hidden by the strong variability in transpirative enrichment. Consequently, $\delta^{18}O$ would be more closely related to tree water status (and thus to $\delta^{13}C$ variation) than to the thermal signal retained in $\delta^{18}O_R$. To assess this hypothesis, we compared the effect of climatic variables on wood $\delta^{13}C$ and $\delta^{18}O$ over the range of environmental conditions where this species can be found. On the other hand, to further test the postulated need for cellulose purification, we aimed to characterise possible changes in the climatic signal present in both $\delta^{13}C$ and $\delta^{18}O$ during the extraction process. The main novelty of our approach lies in the simultaneous examination of both isotopes, as this may help to understand the relationships among the isotope signatures present in different wood components and their implications for environmental studies.

## 2.2. Materials and Methods

### 2.2.1. Plant material and sample preparation

We sampled wood cores (5mm diameter) of *Pinus halepensis* Mill. from 23 sites in the East Iberian Peninsula (Western Mediterranean Basin), selected to be representative of the natural ecological range for this species (see Fig. 2.1). We extracted the cores from the south side of 4-6 adult, dominant trees per aspect (north/south) within each site. Samples were oven-dried at 60ºC for 48h before being polished to allow tree-ring dating. We selected a fragment of the cores, corresponding to the period 1975-1999. Fragments from the same site and aspect (23 sites x 2 aspects = 46) were then pooled and milled to a fine powder.

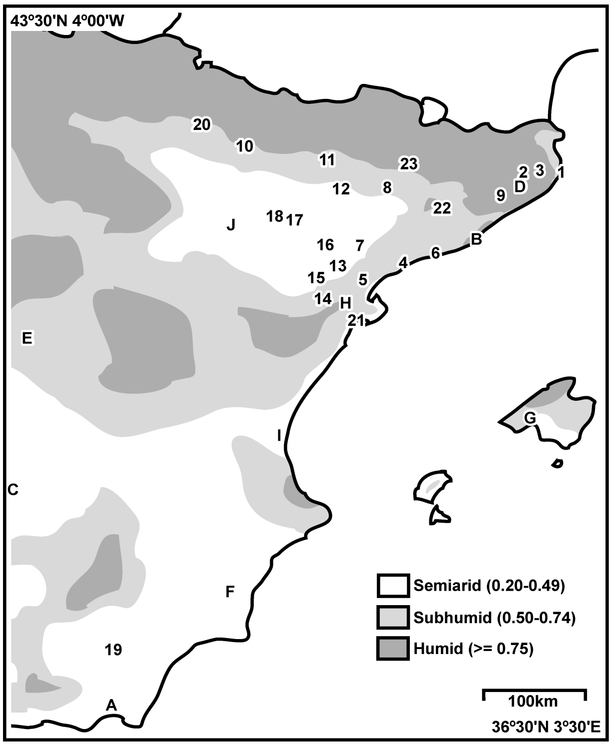

**Fig. 2.1** Geographical distribution of the reference sites (Table 2.1) used to test the accuracy of climatic models and the sampling sites for *Pinus halepensis* wood cores (Table 2.2). Main bioclimatic regions in the studied area are also indicated, according to the UNESCO humidity index (Total precipitation/Pemman evapotranspirative demand). Redrawn from Spanish Ministry of the Environment (2000).

### 2.2.2. Carbon and oxygen isotope analysis

For the removal of extractives and lignin, we adapted the method of Leavitt and Danzer (1993) for holocellulose purification. We divided the samples in three subsets: the first set of samples was analysed for isotope content without previous treatment (whole wood); the second one, after extractives (resins, soluble carbohydrates and other mobile compounds) were removed (extracted wood); and the third one, after the removal of lignin (holocellulose). We weighted 90-110mg dry mass (oven at 60ºC, 24h) within individual pouches made of glass-fiber filter paper (no. 30 Schleicher & Schuell, Dassel). The pouches were cinched up with 4-in nylon cable ties with a number inscribed for identification, and reinforced with waxed flossing tape (dental). We distributed the pouches among 4 soxhlet extractors (250ml) filled with a 2:1 toluene/ethanol mixture and run overnight (16h) at 70ºC. After shutdown and cooling, samples were air-dried for 1-2h before returning them to the soxhlet, filled with 100% ethanol (16h at 70ºC). At this point, samples became free of resins and other solvent-extractable substances. After cooling and air-drying (1-2h), we put the samples in the soxhlet and extracted them with deionised water for 6h at 100ºC. Then we took away half of the pouches ("extracted wood"). The rest of pouches (still wet) were transferred to a 2000ml Erlenmeyer filled with 1000ml deionised water, 5g sodium chlorite ($NaClO_2$, technical grade) and 1.5ml glacial acetic, keeping the flask at 70ºC for 12h. After 4 additions of sodium chlorite and acetic acid, in 12h intervals, we rinsed the samples until the conductivity of the supernatant was less than 0.5 $\mu S\ cm^{-1}$. All samples were oven-dried (60ºC, 48h) and weighted for the quantification of holocellulose. $\delta^{13}C$ and $\delta^{18}O$ in whole wood, extracted wood and holocellulose were determined by mass spectrometry at Isotope Services, Inc., (Los Alamos, NM, USA) and Serveis Científico-Tècnics (Universitat de Barcelona, Spain), respectively. Isotopic composition of lignin was estimated gravimetrically (see e.g. Barbour *et al.* 2001; Borella *et al.* 1998; Gray and Thompson 1977) from the above measurements and the dry-weight ratio between holocellulose and extracted wood ($M_C/M_{eW}$):

**Eq. 2.2**

$$\delta_L = (\delta_{eW} - \delta_C(M_C/M_{eW}))/(1-(M_C/M_{eW}))$$

where $\delta_L$, $\delta_{eW}$ and $\delta_C$ stand for isotopic composition ($\delta^{13}C$ or $\delta^{18}O$) of lignin, extracted wood and holocellulose, respectively.

### 2.2.3. Meteorological data and $\delta^{18}O$ in precipitation

Meteorological data for the period 1975-1999 were supplied by the Instituto Nacional de Meteorología and the Confederación Hidrográfica del Ebro. We took monthly values of precipitation (in mm) and temperature (in ºC). Annual means of $\delta^{18}O$ in rain water ($\delta^{18}O_R$) and vapour pressure have been successfully modelled on a global scale by Barbour *et al.* (2001) from annual precipitation ($P_{an}$), mean annual temperature ($T_{an}$) and altitude (Z, in m). However, annual means are probably imperfect indicators of the great differences in climate seasonality found among Mediterranean sites. Thus, we built alternative models to predict vapour pressure in a monthly basis. We gathered from the Global Network of Isotopes in Precipitation (GNIP) database (IAEA/WMO, 2001) monthly values of precipitation ($P_m$), temperature ($T_m$), vapour pressure (VP) and $\delta^{18}O_R$ from 52 sites across the Mediterranean Region. We calibrated the model using monthly means for each site, and taking only the sites with data available for the whole year. The resulting model for VP is as follows ($r^2$=0.94***, n=312, 26 sites):

**Eq. 2.3**

$$\ln(VP)=6.34+0.047T_m+0.93(P_m/1000)-0.22(Z/1000)$$

We used the data from the sites not included in calibration to validate this model, obtaining a strong correlation between predicted and measured values (r=0.93***, n=102). Moreover, calculating annual means for 10 sites located around the studied area (Fig. 2.1) we obtained better results using Eq. 2.3 than with the global model from Barbour *et al.* (2001; see Table 2.1). Taking monthly means of day-time temperature ($T_{day}$=1/3 average minimum temperature + 2/3 average maximum temperature), we obtained day-time saturation vapour pressure ($VP_{sat}$) on a monthly basis (Jones 1992):

**Table 2.1** Site description and oxygen isotope composition in precipitation ($\delta^{18}O_R$; rainfall-weighted annual mean) for 10 reference sites used to assess the accuracy of vapour pressure ($VP_{an}$, annual mean of vapour pressure) and $\delta^{18}O_R$ models described in Eqs. 2.3 and 2.5. Lat., latitude; Long., longitude; Z, Altitude; $P_{an}$, mean annual precipitation; $T_{an}$, mean annual temperature; $VPD_{an}$, annual mean of day-time vapour pressure deficit; Obs., observed values; Global, predicted values using the global, yearly models developed by Barbour et al. (2001); MED, predicted values using our models for the Mediterranean Region, built on a monthly basis. Correlation coefficients between predicted and observed values are also indicated.

| **Reference Site** | **Lat.** | **Long.** | **Z (m)** | **$P_{an}$ (mm)** | **$T_{an}$ (ºC)** | **$VP_{an}$ (Pa)** | | | **$VPD_{an}$ (Pa)** | | **$\delta^{18}O_R$ (‰)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Obs.** | **Global** | **MED** | **Obs.** | **MED** | **Obs.** | **Global** | **MED** |
| A. Almería | 36º51' | 02º23'E | 21 | 182 | 19.3 | 1503 | 1387 | 1451 | 945 | 825 | -3.7 | -5.0 | -4.3 |
| B. Barcelona | 41º23' | 02º07'E | 90 | 593 | 16.0 | 1347 | 1196 | 1277 | 676 | 757 | -5.0 | -5.7 | -5.1 |
| C. Ciudad Real | 38º59' | 03º55'W | 682 | 383 | 15.9 | 1061 | 1195 | 1125 | 1163 | 891 | -6.9 | -6.7 | -6.4 |
| D. Girona | 41º54' | 02º45'E | 129 | 562 | 15.0 | 1310 | 1142 | 1207 | 650 | 754 | -4.7 | -6.2 | -5.5 |
| E. Madrid | 40º25' | 03º41'W | 655 | 433 | 14.5 | 956 | 1114 | 1051 | 1009 | 915 | -6.4 | -7.1 | -6.4 |
| F. Murcia | 38º00' | 01º10'W | 62 | 287 | 18.9 | 1253 | 1363 | 1439 | 1239 | 988 | -4.6 | -5.1 | -4.6 |
| G. Palma | 39º33' | 02º37'E | 3 | 311 | 18.6 | 1600 | 1346 | 1427 | 779 | 876 | -4.9 | -4.9 | -4.4 |
| H. Tortosa | 40º49' | 00º29'E | 48 | 496 | 18.1 | 1345 | 1320 | 1417 | 1035 | 963 | -4.9 | -5.0 | -4.6 |
| I. València | 39º28' | 00º23'W | 13 | 477 | 18.9 | 1504 | 1366 | 1464 | 902 | 878 | -5.1 | -4.6 | -4.3 |
| J. Zaragoza | 41º39' | 01º01'W | 263 | 301 | 15.8 | 1153 | 1187 | 1207 | 1006 | 969 | -5.5 | -6.4 | -5.2 |
| *r* (pred *vs* obs) | | | | | | | 0.75 | 0.85 | | 0.83 | | 0.75 | 0.85 |
| sig. | | | | | | | ** | *** | | *** | | ** | *** |

**P*<0.05, ***P*<0.01, ****P*<0.001

**Eq. 2.4**

$$VP_{sat}=613.75\times\exp\left[17.502\frac{T_{day}}{240.97+T_{day}}\right]$$

Finally, we estimated monthly means of day-time vapour pressure deficit (VPD) by subtracting VP from $VP_{sat}$.

In the same way, we obtained a monthly model for $\delta^{18}O_R$ ($r^2$=0.52***, n=360, 30 sites):

**Eq. 2.5**

$$\delta^{18}O_R=0.42T_m-0.007T_m^2-26.8(P_m/1000)$$
$$+112(P_m/1000)^2-0.046\sqrt{Z}-8.26$$

On a monthly basis, the estimates of $\delta^{18}O_R$ were less accurate than for VP (r=74***, n=128 for validation sites). Nevertheless, when annual means were calculated, the results obtained with the global model (Barbour *et al.* 2001) were still improved, as shown in Table 2.1.

### *2.2.4. Statistical analysis*

The data were subjected to analysis of variance (ANOVA) to assess the effect of chemical extraction, site and aspect on stable isotope compositions. Principal components analyses (PCA) and simple correlations were used to assess relationships among variables. We built stepwise linear regressions to predict site variables from oxygen and/or carbon isotopes in different wood fractions, but the selection of variables for the model was further restricted in such a way that only the best-fitting wood fraction per isotope (carbon or oxygen) was allowed to enter the model. This was done to quantify the existence of complementary responses to climate variables between carbon and oxygen stable isotopes. Unless otherwise stated, differences were considered statistically significant when *P<0.05*.

## 2.3. Results and discussion

### *2.3.1. $\delta^{13}$C and $\delta^{18}$O values in wood and wood fractions*

As expected, we found significant changes in $\delta^{13}$C and $\delta^{18}$O during removal of extractives and lignin from wood to obtain holocellulose (Table 2.2). On the contrary, we did not find any significant effect of sampling aspect on either $\delta^{18}$O or $\delta^{13}$C, so we decided to work with site means (North and South-facing slopes combined) throughout the study. $\delta^{13}$C of whole wood (δ13CW) and extracted wood ($\delta^{13}C_{eW}$) differed significantly, and both had clearly lower values than that of holocellulose ($\delta^{13}C_C$), with a mean difference of 1.2‰ and 1.0‰, respectively. Unlike for $\delta^{13}$C, there were no differences between $\delta^{18}$O of whole wood ($\delta^{18}O_W$) and extracted wood ($\delta^{18}O_{eW}$), but $\delta^{18}$O of holocellulose ($\delta^{18}O_C$) was about 4‰ higher. Similar results have been reported for $\delta^{13}$C (Borella *et al.* 1998; Leavitt and Danzer, 1993) and $\delta^{18}$O (Barbour *et al.* 2001; Gray and Thompson, 1977) variations in several conifers. The effect of holocellulose purification on $\delta^{13}$C was constant across sites, whereas there was a significant interaction with site for $\delta^{18}$O, probably caused by

**Table 2.2** Site description and mean values (North and South-facing slopes combined) for $\delta^{13}$C and $\delta^{18}$O (‰) in whole wood ($\delta^{13}C_W$, $\delta^{18}O_W$), extracted wood ($\delta^{13}C_{eW}$, $\delta^{18}O_{eW}$), holocellulose ($\delta^{13}C_C$, $\delta^{18}O_C$) and lignin ($\delta^{13}C_L$, $\delta^{18}O_L$). Lat., latitude; Long., longitude; Z, Altitude; $P_{an}$, mean annual precipitation; $T_{an}$, mean annual temperature; $VPD_{an}$, annual mean of day-time vapour pressure deficit; $\delta^{18}O_R$, estimated $\delta^{18}$O of precipitation (rainfall-weighted annual mean).

| **Site** | **Lat.** | **Long.** | **Z (m)** | **$P_{an}$ (mm)** | **$T_{an}$ (ºC)** | **$VPD_{an}$ (Pa)** | **$\delta^{18}O_R$** | **$\delta^{13}C_W$** | **$\delta^{13}C_{eW}$** | **$\delta^{13}C_C$** | **$\delta^{13}C_L$** | **$\delta^{18}O_W$** | **$\delta^{18}O_{eW}$** | **$\delta^{18}O_C$** | **$\delta^{18}O_L$** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 41º56' | 03º15'E | 185 | 693 | 15.2 | 805 | -5.7 | -24.9 | -24.7 | -23.9 | -26.2 | 26.7 | 26.4 | 30.1 | 18.6 |
| 2 | 41º58' | 02º49'E | 44 | 732 | 15.4 | 778 | -5.3 | -24.2 | -24.0 | -23.2 | -25.3 | 27.2 | 27.9 | 30.6 | 23.2 |
| 3 | 42º01' | 03º00'E | 190 | 809 | 14.1 | 723 | -6.0 | -24.7 | -24.4 | -23.6 | -25.4 | 28.1 | 27.7 | 30.3 | 24.2 |
| 4 | 41º09' | 01º17'E | 148 | 478 | 15.8 | 812 | -5.0 | -24.0 | -23.7 | -22.8 | -25.2 | 27.9 | 28.9 | 31.4 | 24.1 |
| 5 | 41º01' | 00º36'E | 180 | 490 | 15.2 | 807 | -5.4 | -23.8 | -23.6 | -22.7 | -25.1 | 28.2 | 28.2 | 31.3 | 22.8 |
| 6 | 41º12' | 01º39'E | 106 | 511 | 15.9 | 849 | -5.0 | -23.8 | -23.7 | -22.7 | -25.2 | 27.1 | 28.2 | 29.7 | 25.9 |
| 7 | 41º20' | 00º30'E | 80 | 395 | 14.8 | 826 | -5.2 | -22.7 | -22.7 | -21.6 | -24.7 | 28.4 | 29.3 | 32.4 | 23.5 |
| 8 | 41º55' | 01º01'E | 525 | 551 | 12.7 | 791 | -6.4 | -24.4 | -24.2 | -23.1 | -25.9 | 28.2 | 29.4 | 31.3 | 26.6 |
| 9 | 41º47' | 02º26'E | 208 | 753 | 15.1 | 807 | -5.8 | -23.7 | -23.7 | -22.7 | -25.1 | 27.0 | 27.9 | 29.5 | 25.7 |
| 10 | 42º19' | 00º45'W | 520 | 519 | 13.7 | 841 | -6.3 | -23.6 | -23.5 | -22.3 | -25.1 | 28.0 | 28.0 | 31.2 | 23.5 |
| 11 | 42º09' | 00º15'E | 625 | 551 | 12.9 | 814 | -6.5 | -24.6 | -24.2 | -23.3 | -25.0 | 28.3 | 28.0 | 31.9 | 24.2 |
| 12 | 41º54' | 00º26'E | 518 | 423 | 12.5 | 788 | -6.3 | -24.0 | -23.8 | -22.7 | -25.2 | 27.5 | 27.1 | 30.9 | 21.4 |
| 13 | 41º10' | 00º23'E | 363 | 427 | 14.7 | 898 | -5.9 | -23.4 | -23.1 | -22.1 | -24.9 | 28.0 | 27.6 | 32.2 | 20.7 |
| 14 | 40º53' | 00º12'E | 630 | 563 | 13.2 | 788 | -6.4 | -24.3 | -24.1 | -23.1 | -25.1 | 27.9 | 27.5 | 31.5 | 23.3 |
| 15 | 41º03' | 00º06'E | 359 | 361 | 15.9 | 997 | -5.3 | -22.8 | -22.7 | -21.7 | -24.8 | 28.1 | 28.8 | 31.7 | 22.8 |
| 16 | 41º23' | 00º17'E | 125 | 385 | 14.5 | 827 | -5.3 | -23.2 | -22.8 | -21.9 | -24.3 | 28.8 | 29.7 | 32.1 | 26.1 |
| 17 | 41º41' | 00º12'W | 356 | 362 | 14.9 | 897 | -5.5 | -23.3 | -22.9 | -22.3 | -24.5 | 27.9 | 28.4 | 31.2 | 23.0 |
| 18 | 41º50' | 00º32'W | 380 | 407 | 13.5 | 801 | -5.8 | -23.2 | -22.7 | -21.6 | -24.0 | 27.7 | 27.5 | 30.7 | 23.7 |
| 19 | 37º26' | 02º20'W | 900 | 337 | 17.8 | 1220 | -5.6 | -22.7 | -22.6 | -21.5 | -24.2 | 28.0 | 28.2 | 34.6 | 18.9 |
| 20 | 42º28' | 01º15'W | 360 | 561 | 13.2 | 743 | -6.2 | -25.0 | -24.9 | -23.7 | -26.5 | 27.2 | 26.4 | 31.5 | 19.6 |
| 21 | 40º41' | 00º34'E | 201 | 562 | 16.7 | 917 | -5.2 | -24.3 | -23.8 | -23.0 | -25.2 | 27.6 | 28.7 | 35.0 | 17.8 |
| 22 | 41º36' | 01º43'E | 230 | 417 | 14.0 | 695 | -5.4 | -23.6 | -23.4 | -22.1 | -25.7 | 27.2 | 27.0 | 30.7 | 20.7 |
| 23 | 42º05' | 01º20'E | 480 | 610 | 13.2 | 773 | -6.2 | -25.0 | -24.4 | -23.4 | -25.8 | 27.1 | 26.7 | 29.6 | 22.7 |
| **Mean** | | | | **517** | **14.6** | **835** | **-5.7** | **-23.9** | **-23.6** | **-22.7** | **-25.2** | **27.7** | **28.0** | **31.4** | **22.7** |
| S.D. | | | | 133 | 1.4 | 106 | 0.5 | 0.7 | 0.7 | 0.7 | 0.6 | 0.5 | 0.9 | 1.4 | 2.4 |

**Table 2.3** Correlation coefficients among site means of $\delta^{13}C$ and $\delta^{18}O$ values for each of the wood fractions studied: whole wood ($\delta^{13}C_W$, $\delta^{18}O_W$), extracted wood ($\delta^{13}C_{eW}$, $\delta^{18}O_{eW}$), holocellulose ($\delta^{13}C_C$, $\delta^{18}O_C$) and lignin ($\delta^{13}C_L$, $\delta^{18}O_L$).

| | $\delta^{13}C_W$ | $\delta^{13}C_{eW}$ | $\delta^{13}C_C$ | $\delta^{13}C_L$ | $\delta^{18}O_W$ | $\delta^{18}O_{eW}$ | $\delta^{18}O_C$ |
|---|---|---|---|---|---|---|---|
| $\delta^{18}O_L$ | 0.12 | 0.10 | 0.08 | 0.21 | 0.34 | 0.51* | -0.48* |
| $\delta^{18}O_C$ | 0.35 | 0.38 | 0.36 | 0.38 | 0.49* | 0.41* | |
| $\delta^{18}O_{eW}$ | 0.54** | 0.54** | 0.46* | 0.52** | 0.73*** | | |
| $\delta^{18}O_W$ | 0.47* | 0.49* | 0.44* | 0.57** | | | |
| $\delta^{13}C_L$ | 0.80*** | 0.87*** | 0.79*** | | | | |
| $\delta^{13}C_C$ | 0.97*** | 0.97*** | | | | | |
| $\delta^{13}C_{eW}$ | 0.98*** | | | | | | |

*$P<0.05$, **$P<0.01$, ***$P<0.001$

the sensitivity of $\delta^{18}O_C$ to climate variables, that was not reflected in $\delta^{18}O_W$ or $\delta^{18}O_{eW}$, as we will discuss in point 2.3.2. On the other hand, our results suggest that, in contrast to $\delta^{13}C$, the sources of variability for $\delta^{18}O$ differed strongly between the main fractions of wood (lignin and holocellulose). δ13C in lignin ($\delta^{13}C_L$) was strongly, positively related to $\delta^{13}C_C$ (Table 2.3) despite being 2.5‰ lower in average. Conversely, $\delta^{18}O_C$ and lignin $\delta^{18}O$ ($\delta^{18}O_L$) differed considerably (8.7‰) and, unlike $\delta^{13}C$ values, were negatively correlated. This observation further evidences that, for carbon and oxygen, the origin of differences in isotope composition between lignin and holocellulose is totally independent. Changes in δ13C may be due to chemical fractionation during lignin biosynthesis (Benner *et al.* 1987) and/or seasonal differences in the amount of carbon directed to the synthesis of cellulose or lignin precursors (Förster *et al.* 2000). However, the main source of variation in $\delta^{13}C$ for $C_3$ plants is shared by all wood components, and it appears to be fractionation during carbon fixation. This explains the observed correspondence between $\delta^{13}C_C$ and either $\delta^{13}C_L$, $\delta^{13}C_{eW}$ or $\delta^{13}C_W$, in agreement with previous works (Borella *et al.* 1998; Mazany *et al.* 1980). On the contrary, the negative correlation between $\delta^{18}O_C$ and $\delta^{18}O_L$ is more controversial, and both negative (Gray and Thompson 1977) and positive (Barbour *et al.* 2001) relationships have been reported. In both studies, $\delta^{18}O_L$ was estimated gravimetrically, as was done in our study, and thus we could discard the errors associated with this method as the cause of such differences. Moreover, such errors would have affected in the same way $\delta^{13}C_L$ and $\delta^{18}O_L$, and they displayed very different behaviours. The most The most probable origin for these contrasting results lies in the exceptional climatic range used by Barbour *et al.* (2001), including over 19‰ of variation in $\delta^{18}O_R$ (1.5‰ in our work). Although the sources of variation for $\delta^{18}O_C$ are well described, little is known about $\delta^{18}O_L$ (Barbour *et al.* 2001). Assuming some exchange of oxygen atoms between xylem water ($=\delta^{18}O_R$) and lignin (as in cellulose), we would expect a positive relationship between $\delta^{18}O_C$ and $\delta^{18}O_L$ over a wide range of $\delta^{18}O_R$, but not if $\delta^{18}O_C$ and $\delta^{18}O_L$ variability can not be explained by $\delta^{18}O_R$, as seems to be our case (see point 2.3.2).

### *2.3.2. Effect of environmental variables on δ13C and δ18O*

A PCA weights plot (Fig. 2.2) provided an initial insight into the association trends for $\delta^{13}C$ and $\delta^{18}O$ along with the environmental variables $P_{an}$, $T_{an}$ and $VPD_{an}$. $\delta^{13}C$ of the different wood fractions clustered together and opposed to $P_{an}$ over the axis of the first component. This agrees with the good correspondence among $\delta^{13}C$ of different wood fractions (Table 2.3) and the expected negative relationship between $\delta^{13}C$ and plant water status. In contrast, the performance of

$\delta^{18}O$ varied substantially among fractions. According to their relative positions in the biplot, $\delta^{18}O_C$ was related to changes in both $VPD_{an}$ and $T_{an}$, whereas $\delta^{18}O$ of other wood constituents was not clearly related to any environmental variable, being relatively independent of $\delta^{18}O_C$ variation. Surprisingly, estimated $\delta^{18}O_R$ was only significantly related to $\delta^{18}O_{eW}$, but not to $\delta^{18}O_C$ (Table 2.4). The same pattern was found when looking at correlations with estimated seasonal means of $\delta^{18}O_R$ (data not shown). However, the highest correlation with $\delta^{18}O_{eW}$ (r=0.49*, N=23) was found in summer, when modelled $\delta^{18}O_R$ showed the poorest correlation with measured $\delta^{18}O_R$ (r=0.49**, ranging from 0.71*** to 0.84*** for the other seasons, N=30). Such relationship might be an artefact, and could be explained by the strong negative relationship found between lignin to cellulose ratio (L/C) and estimated $\delta^{18}O_R$ (Table 2.4). As lignin is isotopically lighter than cellulose, a depletion in lignin content is expected to increase both $\delta^{13}C_{eW}$ and $\delta^{18}O_{eW}$. Indeed, correlation coefficients between $\delta^{18}O_R$ and either $\delta^{13}C_{eW}$ or $\delta^{13}C_W$ were even greater than with $\delta^{18}O_{eW}$. At this point, it should be noted that the relationship between L/C and $\delta^{18}O_R$ has no physiological meaning, being just the consequence of a common influence of temperature and altitude on both variables (Barbour *et al.* 2001; Dansgaard 1964; Kollmann 1955).

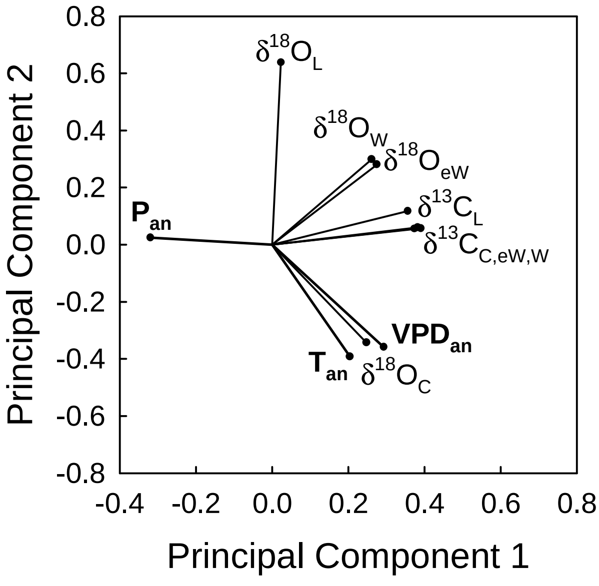

**Fig. 2.2** Component weights plot of the principal components analysis including the three climatic variables studied and site means for $\delta^{13}C$ and $\delta^{18}O$ in whole wood ($\delta^{13}C_W$, $\delta^{18}O_W$), extracted wood ( $\delta^{13}C_{eW}$, $\delta^{18}O_{eW}$), holocellulose ($\delta^{13}C_C$, $\delta^{18}O_C$) and lignin ($\delta^{13}C_L$, $\delta^{18}O_L$). $P_{an}$, mean annual precipitation; $T_{an}$, mean annual temperature; $VPD_{an}$, annual mean of day-time vapour pressure deficit. The two components plotted explained 54% and 19% of total variance, respectively.

The lack of a consistent relationship between d18O of wood constituents and $\delta^{18}O_R$ confirms our hypothesis that, for a species with tight stomatal regulation such as *P. halepensis*, a relatively small range of $\delta^{18}O_R$ (1.5‰) is hidden by the stronger variability in $\Delta^{18}O_e$ (see Eq. 1). The association of $\delta^{18}O_C$ with $VPD_{an}$ (the driving force for transpiration) was stronger than with $T_{an}$ (Table 4), further suggesting that $\delta^{18}O_C$ variations were more closely related to transpirative demand than to the temperature signal stored in $\delta^{18}O_R$. In contrast, other authors found, over a similar range of $\delta^{18}O_R$, good correlations between $\delta^{18}O_R$ and $\delta^{18}O_C$ in tree-rings of *Fagus sylvatica* (Saurer *et al.* 1997b) and *Picea abies* (Anderson *et al.* 1998). However, both species are less adapted to water stress than *P. halepensis*, and thus are expected to show a looser stomatal control. Moreover, Saurer *et al.* (1997b) obtained a poorer relationship with $\delta^{18}O_R$ at the driest site, in agreement with our assumption that the source water signal in $\delta^{18}O_C$ is easily dampened when water is limiting and VPD increases.

$\delta^{13}C$ in the different wood fractions provided similarly strong relationships with climatic variables (Table 2.4). $P_{an}$ showed strong negative correlations with $\delta^{13}C_W$, $\delta^{13}C_{eW}$ and $\delta^{13}C_C$, being somewhat weaker for $\delta^{13}C_L$. On the other hand, $\delta^{13}C$ showed positive trends with $T_{an}$ and $VPD_{an}$. For $T_{an}$, this relationship was only significant for

**Table 2.4** Correlation coefficients between site parameters and either $\delta^{13}C$ or $\delta^{18}O$ in whole wood ($\delta^{13}C_W$, $\delta^{18}O_W$), extracted wood ($\delta^{13}C_{eW}$, $\delta^{18}O_{eW}$), holocellulose ($\delta^{13}C_C$, $\delta^{18}O_C$) and lignin ($\delta^{13}C_L$, $\delta^{18}O_L$), as well as with dry-weight lignin to cellulose ratio (L/C). $P_{an}$, mean annual precipitation; $T_{an}$, mean annual temperature; $VPD_{an}$, mean annual vapour pressure deficit; $\delta^{18}O_R$, estimated $\delta^{18}O$ of precipitation (rainfall-weighted annual mean).

| | **Altitude** | $\mathbf{P_{an}}$ | $\mathbf{T_{an}}$ | $\mathbf{VPD_{an}}$ | $\boldsymbol{\delta^{18}O_R}$ |
|---|---|---|---|---|---|
| $\delta^{13}C_W$ | 0.02 | -0.72*** | 0.43* | 0.56** | 0.45* |
| $\delta^{13}C_{eW}$ | 0.04 | -0.75*** | 0.42* | 0.57** | 0.45* |
| $\delta^{13}C_C$ | 0.11 | -0.78*** | 0.33 | 0.53** | 0.36 |
| $\delta^{13}C_L$ | 0.16 | -0.58** | 0.32 | 0.55** | 0.25 |
| $\delta^{18}O_W$ | 0.17 | -0.46* | -0.04 | 0.25 | -0.03 |
| $\delta^{18}O_{eW}$ | -0.18 | -0.35 | 0.32 | 0.32 | 0.42* |
| $\delta^{18}O_C$ | 0.31 | -0.46* | 0.46* | 0.66*** | 0.17 |
| $\delta^{18}O_L$ | -0.19 | 0.11 | -0.30 | -0.31 | -0.05 |
| L/C | -0.56** | 0.20 | -0.54** | -0.16 | -0.74*** |

*$P<0.05$, **$P<0.01$, ***$P<0.001$

$\delta^{13}C_W$ and $\delta^{13}C_{eW}$, whereas for $VPD_{an}$ we found significant correlations for all wood constituents. As for $\delta^{18}O_R$, the greater sensitivity of whole and extracted wood to $T_{an}$ may be due to the negative relationship observed between L/C and $T_{an}$ (Table 2.4). As lignin is more depleted in $^{13}C$ than the remaining wood, the greater lignin content found in colder sites (see e.g. Kollmann 1955) would have enhanced the depletion in $\delta^{13}C$ associated with lower temperatures (mostly due to lower evaporative demand).

Correlation coefficients between seasonal means of climate variables and $\delta^{13}C$ were also consistent among wood components, despite being weaker for $\delta^{13}C_L$ (Fig. 2.3). Hence, the relationship of $\delta^{13}C$ with precipitation and VPD was significant

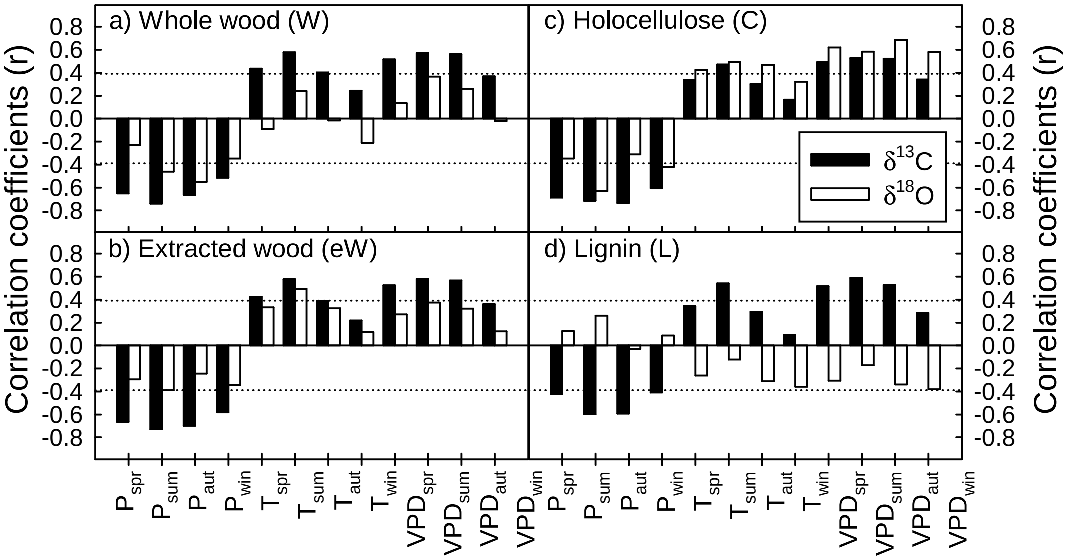

**Fig. 2.3** Correlation coefficients between seasonal means of climatic variables and site means for $\delta^{13}C$ and $\delta^{18}O$ of the different wood fractions. Dotted lines indicate the threshold value for significant correlations ($P<0.05$). P, T and VPD stand for precipitation, temperature and vapour pressure deficit, respectively; each seasonal mean is indicated by the following subindex: $X_{spr}$ (spring), $X_{sum}$ (summer), $X_{aut}$ (autumn) and $X_{win}$ (winter).

throughout the seasons, albeit being a bit lower in winter. For temperature, summer was the most influencing period on $\delta^{13}C$. The higher sensitivity of $\delta^{13}C$ to summer temperature and VPD is a direct consequence of Mediterranean climates, characterized by a dry-hot summer, when VPD strongly determines the water status of plants, and thus $\delta^{13}C$. The generally good sensitivity of $\delta^{13}C$ to precipitation throughout the year may be explained by the particular physiology of the species under study. Unlike in deciduous trees (see e.g. Helle and Schleser 2004), early wood in Pinus species is almost entirely produced from current photosynthate (Dickmann and Kozlowski 1970; Glerum 1980) and both early and late wood isotope composition has proven to be related to the environmental conditions in which they were laid down (Barbour *et al.* 2002; Leavitt and Long 1991). Moreover, *P. halepensis* has an indefinite growth, which often span over the whole year, even though it is slowed during the drier weeks of summer, as well as in cold winters (Borghetti *et al.* 1998; Lev-Yadun 2000; Liphschitz and Lev-Yadun 1986; Nicault *et al.* 2001). Consequently, the isotope composition of tree-rings in this species has the potential of being representative of the environmental conditions throughout the year. However, differences among sites in the seasonal growth pattern might have added an uncontrolled source of variation to the isotopic values of wood material. In order to check for this effect, we calculated the ratio between early and late wood widths in 11 sites (3, 5, 7, 11, 14, 18, 19, 20, 21, 22 and 23; Table 2) where scanned images of the cores were available. Despite having a considerable range of variation for this ratio across sites (1.2-3.5), it was not significantly related to either $\delta^{18}O$ or $\delta^{13}C$ variation. This suggests that growth variability among sites had little influence on the bulk isotopic values obtained.

Unlike for $\delta^{13}C$, holocellulose purification was required to obtain consistent relationships between climatic variables and $\delta^{18}O$. Although $\delta^{18}O_W$ and $\delta^{18}O_{eW}$ were somewhat related to precipitation and temperature, respectively, such relationships were poor and inconsistent (Table 2.4, Fig. 2.3). In contrast, $\delta^{18}O_C$ showed significant relationships with both annual and seasonal means of VPD (r=0.58-0.69), as well as with summer precipitation (r=-0.63). As expected from the lack of correspondence between $\delta^{18}O_C$ and $\delta^{18}O_L$, we did not find any significant correlation between climate variables and $\delta^{18}O_L$. As already suggested by Barbour *et al.* (2001), only along extremely wide ranges of $\delta^{18}O_R$ (unrealistic for most tree-ring studies) it is likely to obtain similar information from holocellulose and wood or lignin. The suggested sources of variability for $\delta^{18}O_L$ are the isotopic composition of atmospheric $O_2$ (used in the synthesis of lignin precursors) and the potential exchange with either leaf or xylem water (Barbour *et al.* 2001). As $\delta^{18}O_L$ and $\delta^{18}O_C$ were negatively correlated, the proportion of oxygen exchanged with water in lignin should have been relatively small; otherwise, they would share the same water-derived $\delta^{18}O$ signal. Thus, the variability in $\delta^{18}O_L$ could be related to variations in $O_2$ isotopic signature. The bulk isotopic value of atmospheric $O_2$ is relatively stable around the world (Barbour *et al.* 2001). However, and despite the high concentration of $O_2$ in the atmosphere, in closed canopies or, at least, within the leaf boundary layer, air $O_2$ isotopic composition is affected by the balance between different respiratory processes with contrasting fractionations for $O_2$ (Angert and Luz 2001; Farquhar and Lloyd 1993; Nagel *et al.* 2001; Ribas-Carbo *et al.* 2000). Probably, such balance is not directly related to the environmental variables here monitored, which would agree with the apparent lack of environmental effects on $\delta^{18}O_L$ variations (Nagel *et al.* 2001). Although the discussion of the mechanisms of $CO_2$ fractionation is far from the aim of this work, further studies to determine the origin of $\delta^{18}O$ signature in lignin are encouraged, as they might offer a new, integrated source of physiological information (e.g. if the isotopic signal from canopy or leaf $O_2$ is somehow preserved in lignin).

### 2.3.3. Are $\delta^{13}C$ and $\delta^{18}O$ complementary?

As a further test of the applicability of combining $\delta^{13}C$ and $\delta^{18}O$ isotopes in environmental studies, we built stepwise regression models to estimate climate variables from oxygen and/or carbon isotopes (Table 2.5). For precipitation, $\delta^{13}C_C$ was the most useful variable, although closely followed by $\delta^{13}C_W$. $\delta^{18}O_C$ was only considered for summer precipitation, where the best model included $\delta^{13}C_W$ and $\delta^{18}O_C$. Conversely, temperature was poorly predicted. For annual means, only $\delta^{18}O_C$ showed some (weak) relationship with temperature. The best fit among temperature models was found in summer, combining $\delta^{13}C_W$ and $\delta^{18}O_C$. On the other hand, VPD was generally related to $\delta^{13}C_W$ and $\delta^{18}O_C$. Indeed, the best predictions were found combining both variables, except in winter, where $\delta^{18}O_C$ was taken alone. In general, $\delta^{13}C_C$ and $\delta^{13}C_W$ showed similar relationships with precipitation, and $\delta^{13}C_W$ was even better related to temperature and VPD, confirming the idea that holocellulose purification is not strictly necessary when assessing $\delta^{13}C$ variation in wood. Nevertheless, for individual tree-ring studies, the use of extracted wood may be still needed, as resins and other extractives are highly mobile, and would add noise to the yearly and/or seasonal climatic signal present in cellulose and lignin. Regarding $\delta^{18}O$, only holocellulose offered a reliable climatic inference. Remarkably, although the isotopic signal of $\delta^{18}O_R$ was apparently lost, $\delta^{18}O_C$ still provided independent information to that reflected in $\delta^{13}C$. This was already evidenced by the poor relationship observed between $\delta^{18}O$ and $\delta^{13}C$ (Table 2.3, Fig. 2.2), further suggesting that the sources of variation for both isotopes differ partially. Hence, $\delta^{13}C$ variability can be mostly explained by the stomatal regulation of $CO_2$ fluxes into the leaf, integrating any environmental variable related to stomatal conductance, e.g. water availability or VPD (Farquhar *et al.* 1982; Ferrio *et al.* 2003b). However, it is also affected by changes in photosynthetic activity derived from irradiance or nutritional stresses, among others (Ferrio *et al.* 2003b; Livingston *et al.* 1998; Yakir and Israeli 1995). On the other hand, $\delta^{18}O$ may also respond to stomatal conductance (see Eq. 1), but it is not affected by photosynthetic activity (Farquhar and Lloyd 1993; Yakir 1992; Yakir and Israeli 1995). Moreover, regardless of its effect on stomatal conductance, VPD has a direct influence on leaf water enrichment as, by definition, it is closely related to the ea term in Eq. 1. Hence, in some cases both isotopes are affected by the same environmental variables (e.g. VPD) but, as the processes underlying such relationships are different, the information derived from each one can still be complementary.

**Table 2.5** Stepwise linear regression models to predict annual and seasonal means of climate variables from $\delta^{13}C$ and/or $\delta^{18}O$ in different wood components. VPD, vapour pressure deficit; $\delta^{13}C_W$, $\delta^{13}C_C$, $\delta^{13}C$ in whole wood and holocellulose, respectively; $\delta^{18}O_C$, $\delta^{18}O$ in holocellulose.

| ***Season*** | **Precipitation (mm)** | | **Temperature (ºC)** | | **VPD (Pa)** | |
|---|---|---|---|---|---|---|
| | **Model** | **$r^2$** | **Model** | **$r^2$** | **Model** | **$r^2$** |
| *Annual* | $-2646 - 139.6*\delta^{13}C_C$ | 0.59*** | $0.1 + 0.46*\delta^{18}O_C$ | 0.17* | $928 + 58.6*\delta^{13}C_W$<br>$+ 41.6*\delta^{18}O_C$ | 0.52*** |
| *Spring* | $-572 - 31.6*\delta^{13}C_C$ | 0.45*** | $31.2 + 0.77*\delta^{13}C_W$ | 0.15* | $684 + 41.7*\delta^{13}C_W$<br>$+ 30.9*\delta^{18}O_C$ | 0.43** |
| *Summer* | $-260 - 28.6*\delta^{13}C_W$<br>$-10.3*\delta^{18}O_C$ | 0.68*** | $33.6 + 0.87*\delta^{13}C_W$<br>$+ 0.31*\delta^{18}O_C$ | 0.37** | $2264 + 110*\delta^{13}C_W$<br>$+ 58.1*\delta^{18}O_C$ | 0.44** |
| *Autumn* | $-967 - 50.2*\delta^{13}C_C$ | 0.52*** | $-1.4 + 0.54*\delta^{18}O_C$ | 0.18* | $815 + 62.5*\delta^{13}C_W$<br>$+ 48.4*\delta^{18}O_C$ | 0.55*** |
| *Winter* | $-483 - 25.9*\delta^{13}C_C$ | 0.34** | - | - | $-652 + 32.8*\delta^{18}O_C$ | 0.31** |

*$P<0.05$, **$P<0.01$, ***$P<0.001$

## 2.4. Conclusions

According to our findings, $\delta^{13}C$ in whole wood may provide similar relationships with climatic variables as those obtained with holocellulose, at least when comparing long-term temporal series from different sites. In contrast, our results point out that, for $\delta^{18}O$, holocellulose purification is indeed necessary to obtain consistent relationships with climatic variables, at least when working with the usual climatic ranges found in tree-ring studies. On the other hand, $\delta^{18}O_C$ in *P. halepensis* was mostly explained by variations in VPD, being unrelated to $\delta^{18}O_R$, confirming our hypothesis that in a species with strong stomatal regulation the signal of source water ($\delta^{18}O_R$) would be lost due to the great variability in evaporative enrichment at the leaf level. Nevertheless, even in such cases, $\delta^{18}O$ may provide climatic information independent from $\delta^{13}C$, as each one reflect in a different way the effect of environmental variables on stomatal conductance and transpirative fluxes. Hence, we found poor correlations between $\delta^{18}O$ and $\delta^{13}C$, which leads to recommend the joint use of $\delta^{18}O$ and $\delta^{13}C$ in environmental studies, as it seems feasible to recover far more information than by implementing both isotopes separately. However, our results also suggest that the particular adaptive properties at the species level strongly determine the kind of information provided by $\delta^{13}C$ and $\delta^{18}O$. Thus, a careful selection of the material of study (e.g. taking co-occurring species with contrasting physiological performance) would probably increase considerably the environmental information obtained from stable isotopes in tree-rings.

### Acknowledgements

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# Chapter 3

*"… le daré verde a los pinos*
*y amarillo a la genista."*

Joan Manel Serrat. *Mediterráneo*

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# Carbon isotope composition of fossil charcoal reveals aridity changes in the NW Mediterranean Basin

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**Abstract** Although several proxies for the inference of precipitation have been proposed, evidence of changes in aridity during the Holocene is scarce, and most is only qualitative. Moreover, precipitation regimes show relatively poor spatial correlations and can exhibit contrasting responses to global climate trends in different areas. Thus, there is a need to concentrate efforts at the local scale in order to increase the spatial resolution of palaeoclimate records, specially regarding water availability in semi-arid zones. Here we propose the analysis of carbon isotope composition ($\delta^{13}C$) in fossil charcoal (routinely recovered from archaeological sites) to quantify changes in water availability in the past. We applied this approach to reconstruct variations in aridity during the last four millennia in the Ebro Depression (NE Iberian Peninsula). First, we studied the effect of carbonization over a range of temperatures (300-500ºC) on the $\delta^{13}C$ of Aleppo pine (*Pinus halepensis* Mill.) wood cores, collected from nine locations in NE Iberian Peninsula with distinct water availability. Despite significant changes in $\delta^{13}C$ caused by carbonization, the original climatic signal of wood $\delta^{13}C$ was well preserved. Moreover, $\delta^{13}C$ shifts induced by this process were successfully corrected by accounting for variation in charcoal carbon concentration (%C). After removing the effect of carbonization, we estimated annual precipitation (P) and the ratio between annual precipitation and evapotranspiration (P/E) from the $\delta^{13}C$ of fossil charcoal. In general, estimated water availability in the past was higher than present values, indicating that latter-day (semi-arid) conditions are mostly due to recent climatic changes. The good agreement between our findings and other evidence indicates that the analysis of $\delta^{13}C$ in charcoal may be useful to expand current palaeoclimatic records as it provides a complementary (and quantitative) source of information to assess climate dynamics.

**Keywords** stable isotopes • palaeoclimate • Late Holocene • proxy • precipitation • water availability • fossil wood • archaeology • *Pinus halepensis* • Iberian Peninsula

## 3.1. Introduction

Global trends in temperature during the Holocene are well documented thanks to a variety of long-term, high-resolution proxy records (see Folland *et al.* 2001). In arid and semi-arid areas, however, precipitation is the most relevant environmental variable that determines the impact of climate fluctuations on ecosystems, agriculture and human settlements. Although several proxies for precipitation have been proposed, most are only qualitative, and evidence of past changes in aridity during the Holocene is still scarce (Folland *et al.* 2001). Moreover, and unlike temperature, precipitation regimes show relatively poor spatial correlations and can exhibit contrasting associations with global climate trends in different areas (see e.g. Rodó *et al.* 1997). This is particularly evident for the Iberian Peninsula (NW Mediterranean Basin), where climate is defined by complex interactions between Atlantic, Mediterranean, Continental and Sub-tropical influences. Indeed, a great spatial heterogeneity in the response to global climatic trends has been reported for the Iberian Peninsula both in present climatic data (Rodó *et al.* 1997) and in the palaeoenvironmental record (Davis 1994; Jalut *et al.* 2000; Magny *et al.* 2003; Riera *et al.* 2004). Therefore, to increase the spatial resolution of palaeoclimatic records, especially regarding water availability in semi-arid zones, supplementary efforts should be concentrated at the local scale.

Carbon isotope composition ($\delta^{13}$C) in wood has been related to several climatic variables that affect plant water availability, and its analysis on tree-rings offers the possibility of high-resolution climate records (see references in Ferrio *et al.* 2003b; Folland *et al.* 2001). However, vast areas of the world have been largely deforested for a long period, thus precluding extensive tree-ring records. To achieve more accurate inferences on past climatic conditions for these areas, complementary sources of plant material in which to perform $\delta^{13}$C analyses, such as charred plant remains recovered at archaeological sites, could be used. This kind of material is relatively common in the archaeological context, and is systematically recovered and identified as it provides information on agricultural practices and ancient landscapes (Van Zeist and Casparie 1984). The use of $\delta^{13}$C analysis in charred crop grains to estimate water availability in early agriculture has already been proposed (Araus and Buxó 1993). This approach provides indirect information on the short period in which the seed is formed, although $\delta^{13}$C may be affected not only by climatic variations but also by changes in crop management practices (Araus *et al.* 1997b; Ferrio *et al.* 2005). Alternatively, charred wood (charcoal) offers the potential to integrate the climatic signal over consecutive years; moreover, it is not directly affected by agricultural practices. Indirect evidence shows that $\delta^{13}$C in fossil charcoal retains the original climatic signature present in the wood (February 2000; Vernet *et al.* 1996). However, the impact of carbonization on the $\delta^{13}$C of wood has not yet been fully described, and the relationship between charcoal $\delta^{13}$C and climate remains unclear. To our knowledge, only one study has addressed $\delta^{13}$C changes during charcoal formation and it reported significantly lower $\delta^{13}$C values for charred material compared to values for intact wood (Jones and Chaloner 1991). The extent of this difference varied depending on the temperature treatment, which in turn affected the degree of carbonization. Therefore, before considering the use of charcoal as a palaeoclimatic indicator, a precise estimation of the degree of carbonization undergone by archaeological remains is required.

Here we analyzed the effect of carbonization on the $\delta^{13}$C of Aleppo pine (*Pinus halepensis* Mill.) wood cores collected from nine locations in NE Iberian Peninsula, which differed in water availability. *P. halepensis* is a typically drought-avoiding, water-saving species that is highly sensitive to changes in water availability, as an adaptive strategy to the large inter-annual and seasonal variability of Mediterranean climates (Ferrio *et al.* 2003a; Liphschitz and Lev-Yadun 1986). Besides, previous studies on this species have shown a strong relationship between several indicators of water availability and $\delta^{13}$C values in wood and its major components, cellulose and lignin (Ferrio *et al.* 2003a; Ferrio and Voltas 2005). Our aim was to assess the degree of preservation of these climatic signals in the $\delta^{13}$C of wood charcoal, and to develop a model to account for the effect (if any) of carbonization. For this purpose, we performed experimental carbonizations over a range of temperatures, and studied the variations in wood $\delta^{13}$C and carbon concentration (%C). We applied the models obtained to a series of archaeological samples from the Mid Ebro Depression (NE Iberian Peninsula) in order to reconstruct the evolution of aridity in this area during the last 4000 years. This region is among the most arid zones in Europe (annual rainfall between 300 and 400 mm); however, it remains unclear whether the present conditions are due to recent environmental changes, or to a progressive aridification which began in prehistoric times. Palaeoenvironmental records for the Late Holocene are still scarce for this area, being mostly derived from pollen and geomorphologic studies (Davis 1994; Gutiérrez-Elorza and Peña-Monné 1998; Riera *et al.* 2004). The application of $\delta^{13}C$ analyses to charcoal might provide complementary (and quantitative) data on water availability in the NW Mediterranean Basin. The information obtained will be discussed in relation to current knowledge about past climates in the region, and in other areas of Europe and the Mediterranean.

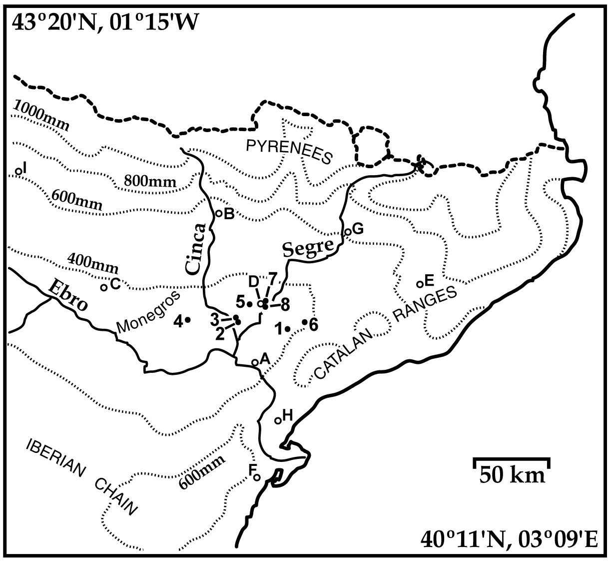

**Fig. 3.1** Geographical distribution of sampling locations for reference wood cores (letters, empty circles), along with archaeological sites where fossil charcoal was collected (numbers, filled circles). Detailed descriptions of sampling locations and archaeological sites are provided in Tables 3.1 and 3.2, respectively.

## 3.2 Materials and methods

### 3.2.1 Reference plant material: tree-ring dating and sample preparation

We sampled wood cores (5.25 mm diameter) of *Pinus halepensis* Mill. from nine locations in NE Spain that covered a representative range of environmental conditions for this species (Fig. 3.1, Table 3.1). Although *P. halepensis* is also found in areas that register up to 800 mm precipitation, we did not include locations with average rainfalls over 700 mm since above this value and according to previous studies in the region (Ferrio *et al.* 2003a) wood $\delta^{13}C$ for this species is mostly insensitive to changes in water availability. We selected two healthy, dominant trees per location, and twin cores were taken from the south side of each. Samples were oven-dried at 60ºC for 48h before being polished to allow tree-ring dating. After visual cross dating, each core was divided in four segments. The number of tree-rings included in the segments was variable (2-6) in order to obtain fragments of similar size (10-20 mm length), but all four cores per location followed identical segment division. A total of 154 segments were obtained (nine locations × two trees × two cores × four segments).

### 3.2.2 Experimental carbonization

For all the analyses, and in order to minimize individual tree effects, we pooled two segments from the same period and location but from distinct trees. Since two cores per tree were taken, for each location we obtained four replicated wood samples, corresponding to four distinct time slices (overall 72 samples). Using direct pair-wise comparisons between treatments, these replicates allowed us to assess the effect of a range of carbonization temperatures on $\delta^{13}C$. To this end, we distributed all samples into four subsets of 18 samples each, which were then subjected to distinct carbonization treatments. In particular, one subset was kept as an untreated control, while the other three were carbonized in a muffle furnace at three maximum temperatures (300ºC, 400ºC, and

**Table 3.1** Main geographical and environmental variables of sampling locations for reference wood cores. Lat., latitude; Long., longitude; Alt., altitude a.s.l.; $P_{an}$, total annual precipitation; $T_{an}$, mean annual temperature; $P/E_{an}$, ratio between $P_{an}$ and total annual evapotranspiration (Thornthwaite 1948).

| Sampling Location | Lat. | Long. | Alt. (m) | $P_{an}$ (mm) | $T_{an}$ (ºC) | $P/E_{an}$ |
|---|---|---|---|---|---|---|
| A. Flix | 41º15’N | 0º32’E | 40 | 389 | 15.9 | 0.46 |
| B. El Grado | 42º10’N | 0º22’E | 520 | 486 | 13.7 | 0.64 |
| C. Leciñena | 41º48’N | 0º28’W | 415 | 368 | 15.2 | 0.44 |
| D. Lleida | 41º37’N | 0º38’E | 220 | 351 | 14.7 | 0.44 |
| E. Montserrat | 41º34’N | 1º47’E | 450 | 387 | 15.4 | 0.47 |
| F. Montsià | 40º40’N | 0º32’E | 200 | 597 | 17.7 | 0.66 |
| G. Oliana | 42º05’N | 1º18’E | 480 | 628 | 13.9 | 0.82 |
| H. Rasquera | 41º01’N | 0º36’E | 135 | 471 | 15.4 | 0.59 |
| I. Sos del R.C. | 42º28’N | 1º15’W | 360 | 609 | 13.2 | 0.84 |

500ºC). The temperatures were chosen to provide material that varied in the degree of carbonization, ranging from slightly charred to fully carbonized (i.e. at the limit of total combustion). All samples reached maximum temperature after a heating ramp of 30 min and they were then kept in the muffle for 30 min. To emulate conditions in which charcoal is formed, we heated the samples under restricted oxygen by burying them in metal crucibles under 3 cm of sand.

### *3.2.3 Archaeological sites and charcoal remains*

169 charcoal remains of *P. halepensis* were recovered from seven archaeological sites in the Segre and Cinca Valleys (Mid Ebro Depression, NE Spain, see Fig. 3.1, Table 3.2) which cover the temporal range between the Bronze Age (*ca.* 2100 BCE) and Modern Age (XVIII c. CE). These sites now display similar climatic and soil conditions, and are characterized by a semi-arid Mediterranean climate. The samples were collected from various archaeological contexts, such as domestic fires, cooking ovens, room floors and levels of rubble from housing structures (for further details about the sites and sampling procedures, see Alonso 1999; Alonso *et al.* 2002). For the sites from the Bronze and First Iron period, radiocarbon ages were determined at Beta Analytical Inc. (Miami, USA) and at the Universitat de Barcelona (Barcelona, Spain), and calibrated dating was calculated with the software CALIB REV 4.3 (Stuiver *et al.* 1998). For the remaining sites, a combination of stratigraphic and archaeological dating was used. To recover plant remains, soil samples were treated using a standard flotation tank in the field with 5 mm, 2 mm and 0.5 mm sieves. For the archaeobotanical analyses, charcoal fragments of at least 5 mm were chosen, and taxonomical determinations were performed on the basis of the anatomical features of the wood. Because of the carbonate enrichment of archaeological sediments, we soaked each charcoal fragment separately with HCl 6M for 24h at room temperature to remove carbonate crusts (DeNiro and Hastorf 1985). The fragments were then rinsed repeatedly with distilled water. Carbonate removal is a crucial step not only to avoid shifts in $\delta^{13}C$, but also to achieve a proper determination of charcoal %C.

### *3.2.4 Carbon concentration and carbon isotope composition*

All samples (reference and archaeological) were oven-dried at 60ºC for 24 hours and milled to fine powder for carbon isotope analyses. $\delta^{13}C$ and %C were determined by elemental analysis and isotope ratio mass spectrometry (EA/IRMS) at Isotope Services, Inc., Los Alamos, NM, USA. To account for changes in $\delta^{13}C$ of atmospheric $CO_2$ ($\delta^{13}C_{air}$) during the Holocene, we calculated carbon isotope discrimination in wood ($\Delta^{13}C_w$) from $\delta^{13}C_{air}$ and wood $\delta^{13}C$ ($\delta^{13}C_w$), as described by Farquhar *et al.* (1982):

**Eq. 3.1**

$$\Delta^{13}C_w = \frac{\delta^{13}C_{air} - \delta^{13}C_w}{(1+\frac{\delta^{13}C_w}{1000})}$$

For extant wood, we obtained $\delta^{13}C_{air}$ for the years included in each core fragment from Francey *et al.* (1999) and the CU-INSTAAR/NOAA-CMDL network for atmospheric $CO_2$ measurements (ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/readme.html). The $\delta^{13}C_{air}$ of the fragments ranged from –7.4‰ to –7.9‰. For archaeological samples, $\delta^{13}C_{air}$ was inferred by interpolating a range of data from the literature which covered the temporal sample range (Eyer *et al.* 2005; Francey *et al.* 1999; Indermühle *et al.* 1999; Leuenberger *et al.* 1992).

### 3.2.5 Meteorological data

Meteorological data was supplied by the Instituto Nacional de Meteorología and the Confederación Hidrográfica del Ebro. We took monthly values for precipitation and temperature within the time period covered by each wood sample used for calibration, and also from 1961 to 1990 for the reference values to characterize present climate at the archaeological sites. From temperature and latitude, potential evapotranspiration was calculated (Thornthwaite 1948). The ratio between precipitation and potential evapotranspiration (P/E) was then calculated as an index of water availability.

### 3.2.6 Statistical analyses

Experimental data were subjected to analysis of variance (ANOVA) to determine the effect of carbonization (fixed factor) and location (random factor) on the $\delta^{13}$C and %C of wood segments. Linear regressions and covariance analyses were calculated to assess the degree of preservation of the climatic signal in $\delta^{13}$C after carbonization, and the relationship between %C and $\delta^{13}$C changes. We fitted locally weighted least squares regression curves (LOESS, span=0.3; (Cleveland 1979) to the data sets to objectively summarize overall trends in the time series of estimated climatic variables. Unless otherwise stated, differences were considered statistically significant when P<0.05.

**Table 3.2** Main geographical and environmental variables (average of present values from the closest meteorological station), dating and cultural period of the archaeological sites. Lat., latitude; Long., longitude; Alt., altitude a.s.l.; $P_{an}$, total annual precipitation; $T_{an}$, mean annual temperature; $P/E_{an}$, ratio between $P_{an}$ and total annual evapotranspiration (Thornthwaite 1948); Calendar year, mean values and dating interval (2$\sigma$ for $^{14}$C calibrated -cal. BCE- dates; estimated range for the rest of sites) for each of the sets of samples studied.

| Archaeological Site | Lat. | Long. | Alt. (m) | $P_{an}$ (mm) | $T_{an}$ (ºC) | $P/E_{an}$ | Calendar year (BCE/CE) |  | Cultural Period |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  | Mean | Interval |  |
| 1. Minferri | 41º30’N | 0º45'E | 240 | 389 | 13.5 | 0.51 | 1890 cal. BCE | 2131-1692 cal. BCE | Bronze Age |
|  |  |  |  |  |  |  | 1673 cal. BCE | 1879-1517 cal. BCE |  |
| 2. Masada de Ratón | 41º29’N | 0º22'E | 130 | 350 | 14.6 | 0.43 | 1262 cal. BCE | 1524-999 cal. BCE | Late Bronze Age |
|  |  |  |  |  |  |  | 1110 cal. BCE | 1185-982 cal. BCE |  |
|  |  |  |  |  |  |  | 1062 cal. BCE | 1048-936 cal. BCE |  |
| 3. Vincamet | 41º30’N | 0º21'E | 110 | 350 | 14.6 | 0.43 | 988 cal. BCE | 1288-928 cal. BCE | Late Bronze Age |
| 4. Tozal de los Regallos | 41º30’N | 0º01'E | 317 | 359 | 14.9 | 0.44 | 896 cal. BCE | 896 cal. BCE | First Iron Age |
| 5. El Vilot de Montagut | 41º38’N | 0º30'E | 250 | 355 | 14.7 | 0.45 | 888 cal. BCE | 1125-795 cal. BCE | Late Bronze Age |
|  |  |  |  |  |  |  | 773 cal. BCE | 801-396 cal. BCE |  |
| 6. Els Vilars | 41º34’N | 0º56'E | 300 | 403 | 14.2 | 0.51 | 488 BCE | 550-425 BCE | Iron Age (Early Iberian) |
| 7. Roques del Sarró | 41º38’N | 0º38'E | 195 | 361 | 14.8 | 0.46 | 255 BCE | 275-175 BCE | Iron Age (Iberian) |
| 8. Ciutat de Lleida | 41º37’N | 0º38'E | 220 | 361 | 14.8 | 0.46 | 26 BCE | 50-1 BCE | Roman |
|  |  |  |  |  |  |  | 67 CE | 20-100 CE |  |
|  |  |  |  |  |  |  | 200 CE | 175-225 CE |  |
|  |  |  |  |  |  |  | 300 CE | 8250-350 CE |  |
|  |  |  |  |  |  |  | 925 CE | 900-950 CE | Middle Age |
|  |  |  |  |  |  |  | 990 CE | 950-1030 CE |  |
|  |  |  |  |  |  |  | 1010 CE | 990-1030 CE |  |
|  |  |  |  |  |  |  | 1025 CE | 950-1100 CE |  |
|  |  |  |  |  |  |  | 1065 CE | 1030-1099 CE |  |
|  |  |  |  |  |  |  | 1600 CE | 1550-1650 CE | Modern Age |
|  |  |  |  |  |  |  | 1750 CE | 1700-1800 CE |  |

## 3.3 Results

### 3.3.1 Effect of carbonization on wood $\delta^{13}C$ and %C

For control (untreated) samples, $\delta^{13}C$ showed considerable variation, ranging from -26.14‰ to -22.63‰ (mean=-24.28‰ ±1.16; herein, mean ± S.D.), whereas %C showed comparatively less variability (48.2% ±0.7). The effect of distinct levels of experimental carbonization (referred to as treatment in Table 3.3) was significant for $\delta^{13}C$ and %C, although it was far stronger for the latter (the treatment factor explained about 28% and 95% of total variation, respectively, for $\delta^{13}C$ and %C). The geographic origin (location) of samples was still the most determinant factor accounting for $\delta^{13}C$ variation (about 48%), while it was not significant for %C. For both variables, the effect of carbonization did not depend on the geographic origin of the samples, as shown by the lack of significant location by treatment interactions. Overall, a significant increase in %C with higher carbonization temperatures was observed, and also a decrease in $\delta^{13}C$ (Fig. 3.2). Although samples treated at 300ºC showed generally lower $\delta^{13}C$ values (-24.50% ±1.12) than controls, they did not differ significantly. Samples treated at 400ºC and 500ºC, however, showed a significantly lower average $\delta^{13}C$ (-25.67‰ ±1.28 and –26.15‰ ±1.61, respectively) than controls and samples treated at 300ºC. Furthermore, mean values and within-treatment variability in %C increased from 50.9% ±0.7 at 300ºC to 70.5% ±1.4 and 77.4% ±5.9 at 400ºC and 500ºC, respectively. Since $\delta^{13}C$ and %C showed similar responses to carbonization, we performed a covariance analysis to determine whether variation in %C could explain changes in $\delta^{13}C$ across treatments. After including %C as a covariable in the ANOVA, the effect of carbonization was no longer significant ($P$=0.601), as this variable explained about 97% of $\delta^{13}C$ variation initially associated with temperature treatments (results not shown).

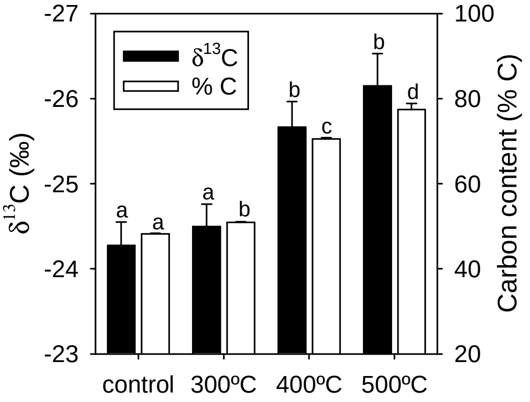

**Fig. 3.2** Mean values and standard error for the $\delta^{13}C$ and %C in intact wood (control) and after carbonization at a range of temperatures. Means with the same letter do not differ significantly on the basis of a least significant difference test ($P$<0.05).

**Table 3.3** Analysis of variance for the effect of carbonization temperature (treatment) and geographic origin (location) in carbon isotope composition ($\delta^{13}C$) and carbon concentration (%C) of wood.

| Source | Sum of Squares | d.f. | Mean Square | *F* ratio | *P* value |
|---|---|---|---|---|---|
| *$\delta^{13}C$* | | | | | |
| Treatment | 44.2 | 3 | 14.8 | 22.9 | 0.000 |
| Location | 76.6 | 8 | 9.6 | 14.7 | 0.000 |
| Treat. x Loc. | 15.6 | 24 | 0.7 | 1.0 | 0.479 |
| Residual | 23.2 | 36 | 0.6 | | |
| *%C* | | | | | |
| Treatment | 11267.7 | 3 | 3755.9 | 492.7 | 0.000 |
| Location | 143.0 | 8 | 17.9 | 2.0 | 0.095 |
| Treat. x Loc. | 217.6 | 24 | 9.1 | 1.2 | 0.312 |
| Residual | 274.4 | 36 | 7.6 | | |

The relationship between $\delta^{13}C$ in wood before and after charring was studied by pair-wise comparisons of those replicated samples (i.e. from the same location and time slice) that were simultaneously tested under control and charring conditions. Despite the changes observed in $\delta^{13}C$ after carbonization, the relationship between intact wood and charcoal $\delta^{13}C$ was strongly significant across the three treatments ($r^2$=0.64, $P$<0.001, N=18). Following the results of the covariance analysis, we performed a multiple linear regression (stepwise) to predict original wood $\delta^{13}C$ ($\delta^{13}C_w$)

using charcoal $\delta^{13}C$ ($\delta^{13}C_{char}$) and charcoal %C (%$C_{char}$, surrogate of the impact of carbonization on $\delta^{13}C_w$) as independent variables. Both variables entered the model, giving the following equation:

**Eq. 3.2**

$$\delta^{13}C_w = -0.706 \times \delta^{13}C_{char} + 0.031 \times \%C_{char} - 8.07$$

$r^2 = 0.72$, $P < 0.001$

The outcome of this model therefore provided estimations of original $\delta^{13}C_w$ from measurements performed on carbonized wood, that is, from $\delta^{13}C_{char}$ and %$C_{char}$. We applied this equation to fossil material to obtain $\delta^{13}C_w$ values directly comparable to those of intact wood (see following sections).

### *3.3.2 $\delta^{13}C$ and %C in archaeological charcoal*

Raw $\delta^{13}C$ values in charcoal ($\delta^{13}C_{char}$) recovered from archaeological sites ranged from -25.39‰ to –20.85‰, with an average of –23.22‰. Mean values per archaeological site and dating are given in Table 3.4. The average %C in charcoals was 59.1%, with values varying from 45.0% to 77.1%. After applying Eq. 2, $\delta^{13}C_w$ showed an average value of –22.64‰ (ranging from –24.38 to

**Table 3.4** $\delta^{13}C$ of wood charcoals recovered from archaeological sites, and present carbonized wood from the three sampling locations closer to the study area. N, number of samples; $\delta^{13}C_{air}$, average $\delta^{13}C$ in atmospheric $CO_2$, according to literature data (see text for details); $\delta^{13}C_{char}$, $\delta^{13}C$ values measured in wood charcoals; %$C_{char}$, carbon concentration, on a dry mass basis; Estim. $\delta^{13}C_w$, estimation of original wood $\delta^{13}C$, following Eq. 3.2; Estim. $\Delta^{13}C_w$, estimated carbon isotope discrimination of original wood, calculated from $\delta^{13}C_w$ and $\delta^{13}C_{air}$, as described in Eq. 3.1.

| **Archaeological Site/Location** | **Calendar Year (BCE/CE)** | **N** | **$\delta^{13}C_{air}$ (‰)** | **$\delta^{13}C_{char}$ (‰)** | **% $C_{char}$** | **Estim. $\delta^{13}C_w$ (‰)** | **Estim. $\Delta^{13}C_w$ (‰)** |
|---|---|---|---|---|---|---|---|
| *Archaeological charcoal* | | | | | | | |
| 1. Minferri | 1890 cal. BCE | 1 | -6.37 | -23.73 | 46.02 | -23.40 | 17.43 |
| 1. Minferri | 1673 cal. BCE | 4 | -6.42 | -22.58 ± 0.60 | 47.72 ± 0.81 | -22.53 ± 0.40 | 16.48 ± 0.41 |
| 2. Masada de Ratón | 1262 cal. BCE | 11 | -6.50 | -23.28 ± 0.26 | 50.01 ± 1.09 | -22.95 ± 0.18 | 16.84 ± 0.18 |
| 3. Vincamet | 1110 cal. BCE | 8 | -6.51 | -23.62 ± 0.14 | 62.57 ± 2.16 | -22.81 ± 0.12 | 16.67 ± 0.13 |
| 2. Masada de Ratón | 1062 cal. BCE | 5 | -6.52 | -23.28 ± 0.30 | 56.77 ± 3.15 | -22.74 ± 0.27 | 16.60 ± 0.28 |
| 2. Masada de Ratón | 988 cal. BCE | 8 | -6.53 | -23.04 ± 0.10 | 52.93 ± 1.67 | -22.70 ± 0.06 | 16.54 ± 0.06 |
| 4. Tozal de los Regallos | 896 cal. BCE | 28 | -6.54 | -23.52 ± 0.17 | 62.49 ± 1.26 | -22.74 ± 0.14 | 16.58 ± 0.14 |
| 5. El Vilot de Montagut | 888 cal. BCE | 8 | -6.54 | -22.79 ± 0.23 | 58.77 ± 2.82 | -22.34 ± 0.21 | 16.16 ± 0.22 |
| 5. El Vilot de Montagut | 773 cal. BCE | 9 | -6.54 | -22.85 ± 0.15 | 54.97 ± 2.47 | -22.49 ± 0.16 | 16.32 ± 0.17 |
| 6. Els Vilars | 488 BCE | 8 | -6.49 | -23.23 ± 0.13 | 57.52 ± 2.89 | -22.69 ± 0.12 | 16.57 ± 0.13 |
| 7. Roques del Sarró | 225 BCE | 11 | -6.45 | -22.37 ± 0.40 | 58.04 ± 2.07 | -22.06 ± 0.32 | 15.97 ± 0.34 |
| 8. Ciutat de Lleida | 26 BCE | 9 | -6.42 | -23.80 ± 0.21 | 59.75 ± 2.35 | -23.02 ± 0.15 | 17.00 ± 0.15 |
| 8. Ciutat de Lleida | 67 CE | 6 | -6.40 | -24.13 ± 0.43 | 63.62 ± 4.43 | -23.13 ± 0.43 | 17.13 ± 0.45 |
| 8. Ciutat de Lleida | 200 CE | 2 | -6.37 | -21.80 ± 0.03 | 59.12 ± 0.10 | -21.63 ± 0.02 | 15.60 ± 0.02 |
| 8. Ciutat de Lleida | 300 CE | 10 | -6.36 | -23.50 ± 0.12 | 59.36 ± 1.94 | -22.82 ± 0.08 | 16.85 ± 0.08 |
| 8. Ciutat de Lleida | 925 CE | 9 | -6.43 | -22.71 ± 0.40 | 61.22 ± 1.78 | -22.20 ± 0.30 | 16.14 ± 0.31 |
| 8. Ciutat de Lleida | 990 CE | 6 | -6.42 | -23.66 ± 0.35 | 60.06 ± 2.47 | -22.91 ± 0.29 | 16.88 ± 0.30 |
| 8. Ciutat de Lleida | 1010 CE | 10 | -6.42 | -23.42 ± 0.13 | 61.32 ± 1.96 | -22.70 ± 0.12 | 16.66 ± 0.12 |
| 8. Ciutat de Lleida | 1025 CE | 6 | -6.43 | -22.44 ± 0.48 | 62.07 ± 2.14 | -21.98 ± 0.33 | 15.90 ± 0.34 |
| 8. Ciutat de Lleida | 1065 CE | 2 | -6.43 | -23.03 ± 0.18 | 57.54 ± 0.14 | -22.54 ± 0.12 | 16.48 ± 0.12 |
| 8. Ciutat de Lleida | 1600 CE | 3 | -6.31 | -22.99 ± 0.02 | 64.66 ± 0.88 | -22.29 ± 0.04 | 16.35 ± 0.04 |
| 8. Ciutat de Lleida | 1750 CE | 5 | -6.33 | -23.80 ± 0.33 | 66.93 ± 0.31 | -22.80 ± 0.23 | 16.85 ± 0.24 |
| *Present charcoal* | | | | | | | |
| A. Flix | 1985 CE | 6 | -7.60 | -23.60 ± 0.35 | 63.97 ± 4.45 | -22.75 ± 0.24 | 15.50 ± 0.24 |
| C. Leciñena | 1992 CE | 6 | -7.75 | -24.64 ± 0.18 | 66.32 ± 4.84 | -23.41 ± 0.10 | 16.04 ± 0.11 |
| D. Lleida | 1988 CE | 6 | -7.67 | -24.14 ± 0.48 | 64.57 ± 4.68 | -23.11 ± 0.25 | 15.81 ± 0.27 |

–20.86). Carbon isotope discrimination ($\Delta^{13}C_w$), calculated from estimated $\delta^{13}C_w$ and $\delta^{13}C_{air}$, ranged from 14.74‰ to 18.38‰, with an average of 16.55‰. Overall, the archaeological material exhibited higher $\Delta^{13}C_w$ values than samples taken from the three locations closer to the area of study and used in the carbonization experiment (average value of 15.79‰, Table 3.4). For most of the archaeological sites, the variability in $\Delta^{13}C_w$ was similar to that found in present-day wood charcoal (average within-site standard deviation of 0.71‰ and 0.61‰, for archaeological sites and sampling locations, respectively).

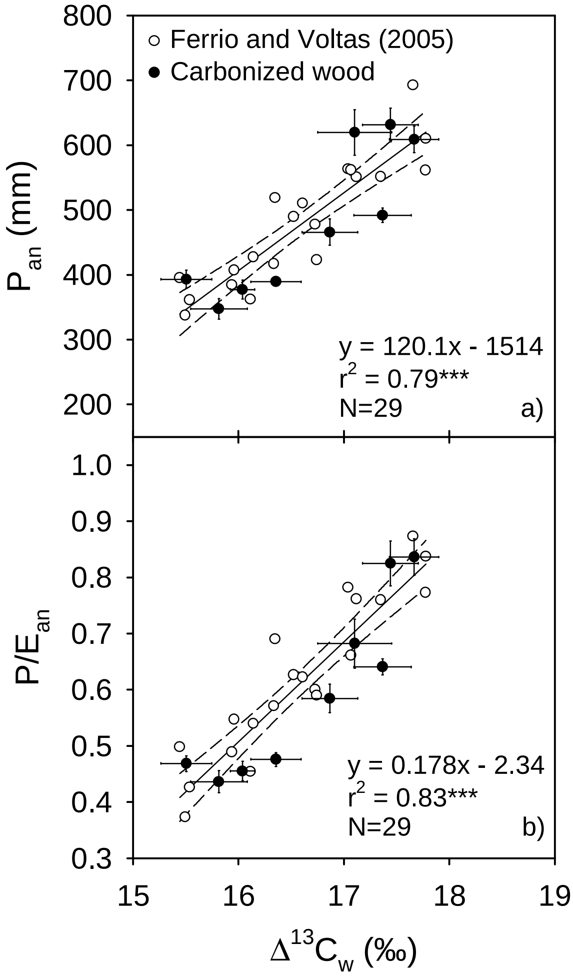

**Fig. 3.3** Relationship between carbon isotope discrimination in wood ($\Delta^{13}C_w$) and either a) annual precipitation ($P_{an}$) or b) the ratio between $P_{an}$ and evapotranspiration ($P/E_{an}$). Empty circles, intact wood values, as reported by Ferrio and Voltas (2005); filled circles, estimated $\Delta^{13}C_w$ from the analysis of experimentally carbonized wood, as described in Eq. 3.2. Error bars indicate standard errors for the means.

### *3.3.3 Estimation of past water availability from the $\Delta^{13}C$ of archaeological charcoal*

To assess the implications of $\delta^{13}C$ analysis on charcoals for palaeoclimatic reconstruction, we also applied Eq. 2 to the wood segments that had been experimentally carbonized, but for which direct information on intact wood $\delta^{13}C$ values was not available. Estimates of $\delta^{13}C_w$ (from $\delta^{13}C_{char}$ and $\%C_{char}$) were used to calculate $\Delta^{13}C_w$ values, which were applied to infer climatic variation across the reference locations (nine). No significant relationship between mean annual temperature ($T_{an}$) and estimated $\Delta^{13}C_w$ was detected. On the contrary, both total annual precipitation ($P_{an}$) and the ratio between $P_{an}$ and total evapotranspiration ($P/E_{an}$) were consistently correlated with $\Delta^{13}C_w$ across locations for each temperature treatment (r=0.61 to 0.96, $P<0.05$-$P<0.001$). Moreover, neither the slope nor the intercept of these relationships differed significantly between treatments, according to an heterogeneity of slopes ANOVA. Consequently, we combined $\Delta^{13}C_w$ values across treatments to obtain more robust $\Delta^{13}C_w$ averages for each location. These values are plotted in Fig. 3.3, together with those reported by Ferrio and Voltas (2005) for intact wood of *P. halepensis* covering a similar ecological gradient (20 locations). The resulting relationships between $\Delta^{13}C_w$ and either $P_{an}$ or $P/E_{an}$ were strongly significant. The slope and intercept terms of the regression lines did not differ significantly from those reported in Ferrio and Voltas (2005) for intact wood data.

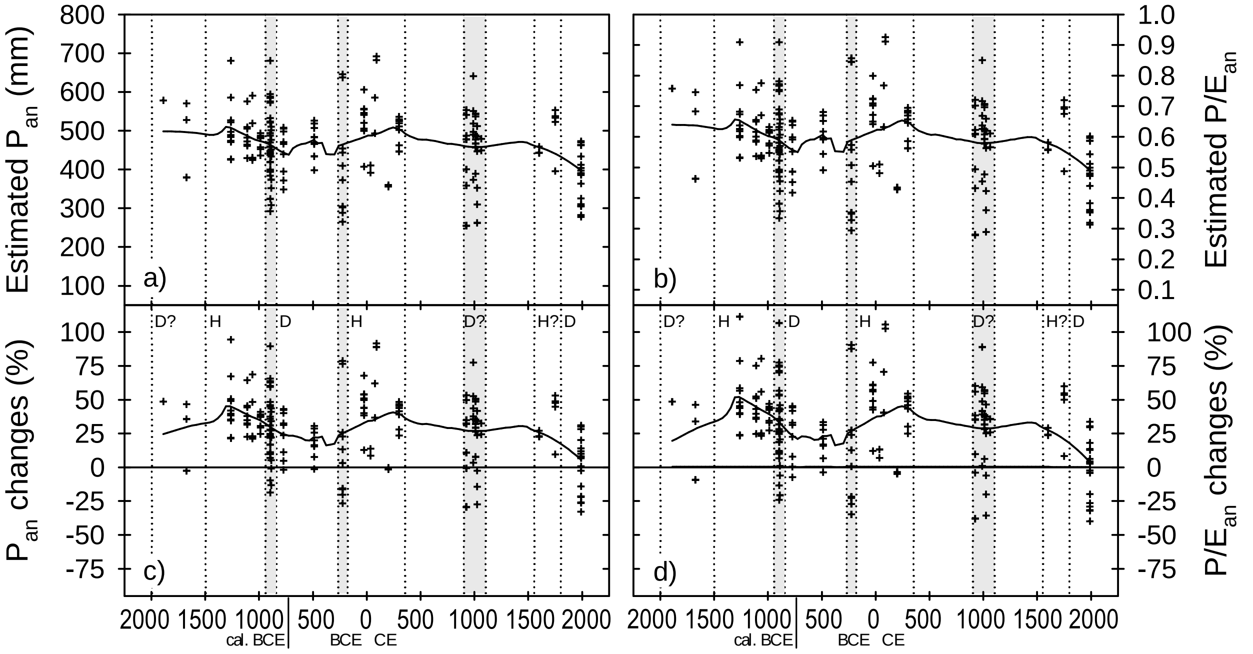

**Fig. 3.4** Estimated evolution of annual precipitation ($P_{an}$; a, c) or the ratio between $P_{an}$ and evapotranspiration ($P/E_{an}$; b, d), according to the estimated $\Delta^{13}C_w$ values plotted in Table 3.4. $P_{an}$ and $P/E_{an}$ changes, % change relative to present climate data. Trend lines depict locally weighted least squares regression curves (LOESS, *span*=0.3) fitted to the data. Relatively dry (D) and humid (H) phases are indicated. Periods of strong climatic variability are highlighted (shadow areas).

Models from Fig. 3.3 were subsequently applied to estimated $\Delta^{13}C_w$ values of archaeological charcoal in order to infer past trends in water availability (Fig. 3.4a,b). Estimated $P_{an}$ ranged from 255 to 692 mm (472 mm in average), whereas $P/E_{an}$ varied between 0.28 and 0.93, with an average value of 0.60. In both cases, estimated values for archaeological samples were significantly higher than the values recorded for present times, averaged across archaeological sites ($P_{an}$=361 mm, $P/E_{an}$=0.45). They were also higher than estimations derived from $\delta^{13}C$ values of present-day wood collected in the same area (Ebro Depression, locations A, C and D, see Table 3.4) and experimentally carbonized (estimated $P_{an}$=381 mm and $P/E_{an}$=0.47). To avoid possible artifacts in the comparison with present conditions arising from differences in the geographic origin of archaeological samples, we also expressed estimated values as percentage of change with respect to current climatic values at each archaeological site (Table 3.2, Fig. 3.4c,d). Mean values of $P_{an}$ and $P/E_{an}$ were predominantly above present-day values, with an average increase of about 31% and 34%, respectively. We detected two main phases of high $P_{an}$ and $P/E_{an}$ (1500-900 BCE; 300 BCE-300CE), alternating with phases of lower water availability, relatively closer to current conditions (2000-1500 BCE; 900-300 BCE). Finally, a period of strong climatic variability was identified from the tenth to the eleventh century, followed by relatively humid conditions from the sixteenth to the eighteenth century.

## 3.4 Discussion

### 3.4.1 Is the climatic signal of wood $\delta^{13}C$ preserved in charcoal?

The two major components in wood, lignin and cellulose, differ substantially in their isotopic composition, the former showing a lower $\delta^{13}C$ than the latter (Borella *et al.* 1998; DeNiro and Hastorf 1985; Ferrio and Voltas 2005). Moreover, cellulose is more rapidly combusted than lignin (Beall *et al.* 1974; Kollmann 1955), thus causing substantial isotopic depletion of charcoal when compared to intact wood (Jones and Chaloner 1991). Nevertheless, since $\delta^{13}C$ displays similar responses to climate in cellulose and lignin (Borella *et al.* 1998; Ferrio and Voltas 2005), this fractionation during charring would not override the climatic signal of charcoal $\delta^{13}C$. In fact, and despite considerable changes in $\delta^{13}C$ during carbonization, our results reveal that the environmental signature of wood $\delta^{13}C$ is still present in charcoal. This can be derived not only from the persistence of a geographic signature across a range of heat treatments, but also from the strong correlation between $\delta^{13}C$ in intact and carbonized wood. Indeed, we obtained a model to estimate the original $\delta^{13}C$ of wood (i.e. before carbonization) from charcoal $\delta^{13}C$, including %C as an indicator of the degree of carbonization. Using this model, we found consistent relationships between $\Delta^{13}C$ of carbonized wood (corrected for the effect of carbonization) and climatic variables, and these relationships did not differ significantly from those previously reported for intact wood of *P. halepensis* (Ferrio *et al.* 2003a; Ferrio and Voltas 2005). Our methodology can be easily implemented in $\delta^{13}C$ studies in charcoal, as it does not require further measurements (%C is routinely included in carbon isotope analyses). Nevertheless, as the $\delta^{13}C$ changes observed during carbonization are mostly attributable to differential combustion of lignin and cellulose, and their amounts can differ among species (Kollmann 1955), this particular correction model might not be directly applicable to other species, mainly non-conifers.

### 3.4.2 %C in charcoal as indicator of carbonization level

As expected, %C had a low range of variation in intact wood, even in trees grown under contrasting environmental conditions. Indeed, this variable shows a rather constant value of around 50%, regardless of the species, environmental conditions or type of wood (Kollmann 1955). As %C increases progressively during charring, it is a good indicator of the degree of carbonization, which can be potentially useful to correct d13C shifts related to this process. However, it should be noted that the range of variability of %C increased considerably with increasing temperature levels. The most probable explanation for this effect would be that the heat released during combustion further increased the effective temperature of carbonization, particularly at the highest temperatures. This process, known as self-combustion, is enhanced by resins and other volatiles, and thus may differ from one piece of wood to another (Kollmann 1955). Similarly, this process would explain the greater spread of d13C values for the highest temperature treatment.

The average %C in archaeological samples (59.1%) indicates that most of the samples reached intermediate carbonization levels between the 300ºC and 400ºC treatments. This observation is consistent with evidence from anatomical studies and spectral measurements (Guo and Bustin 1998; Jones and Chaloner 1991), thereby indicating that most of the fossil charcoal was formed between *ca.* 350ºC and *ca.* 450ºC. This “optimal” temperature range is probably the result of the two critical requirements for the preservation of fossil plant remains. They must become inert enough to prevent microbial decomposition, while retaining some physical resistance to avoid destruction during burial and post-burial processes, including recovery (Ferrio *et al.* 2004; Jones and Chaloner 1991; Van Zeist and Casparie 1984; Wright 2003).

### 3.4.3 Changes in aridity during the last 4000 years in the NW Mediterranean Basin

In spite of the aforementioned constraints, our approach provided a robust quantification of climatic variables directly associated with water availability, such as $P_{an}$ and $P/E_{an}$. This methodology makes the comparison between past and present-day conditions easier than the most common qualitative or semi-quantitative approaches (see references in Folland *et al.* 2001). In our study, estimated $P_{an}$ and $P/E_{an}$ from the analysis of charcoal $\delta^{13}C$ were significantly higher in the past than in present times, and showed an increase over 30% in relation to current values. These results were supported by $\delta^{13}C$ analysis of experimentally carbonized wood from three reference locations (A, C and D in Table 3.1), as these sites provided significantly lower estimations of $P_{an}$ and $P/E_{an}$ than the average for archaeological samples. Moreover, the good agreement between recorded and estimated values across the three reference locations further supports the validity of our models (estimated $P_{an}$=381 mm and $P/E_{an}$=0.47, climatic data $P_{an}$=379 mm and $P/E_{an}$=0.48).

On the basis of these findings, present-day semi-arid conditions in the study area may be mostly due to recent climatic shifts towards a harsher climate, probably enhanced by anthropogenic disturbance. A considerable decrease in precipitation in NW Mediterranean has been reported after 1850, as shown by tree-ring analyses (Creus *et al.* 1996), lake-level records (Riera *et al.* 2004) and documentary studies (Barriendos and Martín-Vide 1998). In addition to a fall in precipitation, the global increase in temperature since the mid XIXth century have augmented plant water demand, and, consequently, water stress, throughout the Mediterranean Region (Creus *et al.* 1996; Folland *et al.* 2001).In addition to the overall differences observed between past and present-day values, two main phases of greater water availability alternating with drier periods were detected. The first humid phase (1500-900B CE) gave significantly higher averages of $P_{an}$ and $P/E_{an}$ than those for the present (increase of 36% and 42%, respectively). This humid period coincided with the interphase between two aridification periods (2350-1450BCE and 900BCE-220 CE) as defined by Jalut *et al.* (2000) for NW Mediterranean on the basis of a transect of pollen analyses. Because of the small number of samples available, the relatively arid episode described by Jalut *et al.* (2000) around 2350-1450BCE was not well characterized in our data. Nevertheless, we found a trend towards lower water availability from 2000BCE to 1500BCE (when compared to the following period) although, on average, the $P_{an}$ and $P/E_{an}$ estimated for this period were still higher than present-day values.

The most consistent arid phase within the timeframe of this study corresponded to the period 900-300BCE. Conversely, even in this phase, our estimates of water availability were significantly higher than for present times (about 21%). This period was followed by a recovery of water availability between 300 BCE and 300 CE, with average $P_{an}$ and $P/E_{an}$ about 33% and 36% higher than present-day values. The analysis of $\delta^{13}C$ in charcoals from a Mediterranean deciduous oak (*Quercus pubescens* W.) performed by Vernet *et al.* (1996) in Southern France also indicated a period of lower water availability *ca.* 3000-2700 BP, a subsequent recovery *ca.* 2200-1600 BP, and a declining trend onwards until present times. Estimated water inputs of archaeological cereal crops (wheat and barley) grown in NW Mediterranean (Araus and Buxó 1993) are also consistent with these general trends. Furthermore, pollen analyses performed at the archaeological site of El Vilot de Montagut (Alonso *et al.* 2002) reveal an increase in aridity at the beginning of the 3rd millennium, which is in agreement with the low $\Delta^{13}C$ values found for this site. Likewise, the humid phase between 300 BCE and 300 CE was also confirmed by pollen data from the city of Lleida (0-200 CE), which indicated a relatively wet climate (Riera S., unpublished results). Similarly, lower groundwater fluxes from 500 to 100 BCE than between 100 BCE and 200 CE have been reported (Magny and Richard 1992). Some studies, however, provide apparently contradictory results for the arid period 900-300 BCE. Geomorphologic studies throughout the Ebro Basin (Gutiérrez-Elorza and Peña-Monné 1998) report a generalized increase in sediment accumulation on slope deposits around 1000-400 BCE, which was interpreted as an increase in vegetation cover, and a colder/wetter climate. Gribbin and Lamb (1978) had previously described a decrease in temperature of about 2ºC for this period, which was further supported by an expansion of the glaciers, and evidence of a colder climate in North and Central Europe (see e.g. Magny *et al.* 2003). These findings are partly supported by vegetation reconstructions (based on charcoal analyses) from the Cinca and Segre Valleys (Alonso 1999), which show the presence of mixed forests of holm oaks, pines and deciduous oaks (i.e. colder/wetter climate than today). The most probable explanation for such divergent results might be found in the distinct physiological responses of *P. halepensis* to temperature and water regimes. An abrupt decrease in winter temperatures would have shortened the growing cycle of this species, as it is sensitive to cold and can virtually arrest growth during the cold season (Liphschitz and Lev-Yadun 1986). In addition, although lower temperatures are expected to reduce water demand in summer, they might also have reduced the incidence of convective storms (and thus water availability) from late spring to early autumn (Gribbin and Lamb 1978). Indeed, $\Delta^{13}$C analyses of cereal grains (Araus *et al.* 1997a; Araus and Buxó 1993) indicate that water inputs during grain filling (late spring) decreased significantly during this period in NW Mediterranean. Moreover, in the nearby Monegros region, charcoal studies show a decrease in vegetation cover, and also an increase in continentality, thereby leading to the disappearance of cold-sensitive species (Alonso 1999). Consequently, a combination of colder/wetter winters, but drier summers, might have caused an overall increase in water stress for *P. halepensis* during this period. This hypothesis would be fully compatible with a reduction of soil erosion (, enhanced by the absence of convective storms, without discarding an increase in vegetation cover, driven by cold-resistant species with deep-root systems to exploit winter-fed water reservoirs, e.g. Mediterranean oaks (Alonso 1999). Expanding $\Delta^{13}$C analyses to these species might help to elucidate the origin of these contrasting results, and provide useful information on the seasonality of precipitation events.

We identified three periods of strong climatic variability (shaded areas in Fig. 3.4: 900-800 BCE; 275-175 BCE; 900-1100 CE), the first two coinciding with the transition between the aforementioned phases of higher and lower water availability. According to our data, the third period was characterized by relatively low and unstable $P_{an}$ and $P/E_{an}$ values (but still 26-28% above current values). Because of the lack of samples dating immediately before and after the 900-1100 CE, we cannot attribute the great variability of this period to a “transition effect”. This phase coincides with the Medieval Warm Period (Gribbin and Lamb 1978), dated between 1000 and 1200 CE, and characterized by an overall increase in temperature. According to those authors, an increase in summer temperatures would cause higher water deficits, and the importance of convective storms would be increased, which is consistent with the dry and unstable climatic conditions reported here. Pollen data from the city of Lleida also indicate drier conditions in this period than at the beginning of the 1st Millennium CE (Riera S., unpublished results). Documentary studies for this period reflect a climate with strong interannual contrasts, alternating drought periods with catastrophic floods (Gargallo 1989). Furthermore, soil erosion increased around 400-1200 CE ((Gutiérrez-Elorza and Peña-Monné 1998), which is consistent with a more arid and variable climate. Our analyses revealed a subsequent recovery in water availabity from the XVIth to the XVIIIth century (35-38% over current values), although, because of the small number of samples available, these results are not conclusive. Nevertheless, this period coincides with the Little Ice Age (1450-1850 CE), which is characterized by strong climatic variability (Barriendos and Martín-Vide 1998; Creus *et al.* 1996; Riera *et al.* 2004), lower mean temperatures (Creus *et al.* 1996; Gribbin and Lamb 1978), reduced erosion (Gutiérrez-Elorza and Peña-Monné 1998) and increased lake levels (Riera *et al.* 2004).

## 3.5 Conclusions

Our experimental data demonstrates that the climatic signal of wood $\delta^{13}C$ is preserved after carbonization, and that shifts in $\delta^{13}C$ caused by this process can be successfully removed. Moreover, the generally good agreement between our findings and evidence provided by several palaeoenvironmental data from NW Mediterranean supports the usefulness of $\delta^{13}C$ analysis in wood charcoal to expand current palaeoclimatic records, especially in dry areas where other records (e.g. lake sediments, ice-cores, tree-rings) are relatively scarce. Even in regions where long palaeoclimatic records are available, $\delta^{13}C$ in charcoal might provide a complementary (and quantitative) source of information, in order to achieve a greater understanding of past climatic dynamics. The divergences between our results and some palaeoenvironmental evidence, however, highlight the complexity of combining information from distinct sources. Nevertheless, interdisciplinarity is an essential prerequisite for palaeoenvironmental studies, as each proxy has its own constraints, which can be overcome only by considering alternative inferential sources. In this regard, the particular physiological characteristics of the species under investigation must be considered to ensure a proper interpretation of $\delta^{13}C$ trends in charcoal.

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# Chapter 4

*"Le blé pour moi est inutile. Les champs de blé ne me rappellent rien. Et ça, c'est triste!"*

Antoine de Saint Exupéry. *Le Petit Prince*

Chapter 4

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# Water management practices and climate in ancient agriculture: inference from the stable isotope composition of archaeobotanical remains

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**Abstract** Carbon isotope discrimination ($\Delta^{13}C$) in charred grains from archaeological sites provides reliable information about water availability of ancient crops. However, as cereals are cultivated plants, they may reflect not only climatic fluctuations, but also the effect on water status of certain agronomic practices, such as sowing in naturally wet soils or irrigation. In this work, we propose a methodological approach to combine $\Delta^{13}C$ data from different plant species, in order to discriminate between climate-derived and anthropogenic effects on ancient crops. We updated previous models to estimate water inputs from $\Delta^{13}C$ of cereal grains (Hordeum vulgare and Triticum aestivum/durum), and we applied them to compiled literature data from several archaeological sites, including samples from Neolithic to present-day in NE and SE Spain, as well as from the Neolithic site of Tell Halula (NW Syria). We found an important decrease in water availability from Neolithic to present-time in the three areas of study, specially clear for the two driest areas (SE Spain and NW Syria). Potential differences in water management practices between wheat and barley, as well as between cereal and legume crops (Vicia faba and Lens culinaris), were also discussed, based on the comparison of $\Delta^{13}C$ values across several archaeological sites.

**Keywords** Carbon isotope discrimination ($\Delta^{13}C$) • wheat • barley • legumes • Middle Euphrates • Iberian Peninsula

## 4.1. Introduction

Improving our knowledge about the way early farmers reacted to environmental changes might assist to face current conflicts resulting from water shortage in different areas of the world. Up to now, the most evident way to get insight into ancient water management methods is the study of archaeological structures related to water uptake or distribution. However, this methodology is limited to relatively advanced societies, and does not give any information about the actual results of such management on crops performance. Several indirect methods have been proposed to determine the water status of ancient crops, such as the size of charred seeds (Ferrio *et al.* 2004; Helbaek 1960), the analysis of phytoliths (Rosen and Weiner 1994), or the study of weed flora (Jones *et al.* 1995). However, none of these methods is conclusive, and some of them, such as phytolith analyses, are only applicable to certain crops.

Carbon isotope composition ($\delta^{13}C$) in $C_3$ plant tissues constitutes an integrated record of the ratio of intercellular to atmospheric concentration of $CO_2$ during the period in which the carbon was fixed, and thus reflects the balance between carbon fixation and stomatal conductance. Plants typically react against a decrease in water availability through stomata closure and, thus, $\delta^{13}C$ from plant tissues is a good indicator of the water status during the time these tissues were formed (see Farquhar *et al.* 1989 for further details on carbon isotope theory). Many studies under growth-chamber and field conditions have shown that plants developed under water stress produced leaves with higher (i.e. less negative) $\delta^{13}C$ values (see references in Ferrio *et al.* 2003b; Hubick *et al.* 1993). According to these findings, a significant relationship between $\delta^{13}C$ and environmental parameters related to water availability would be expected. Although most of the basic studies on $\delta^{13}C$ and plant water relations have been performed on leaf material (as this is the tissue directly involved in photosynthesis), further works has shown that similar relationships can be established using other plant tissues such as seeds or wood.

Wood $\delta^{13}C$ has been related to changes in various climatic variables, including humidity (Saurer and Siegenthaler 1989; Stuiver and Braziunas 1987), precipitation (Ferrio *et al.* 2003a; Warren *et al.* 2001) and water pressure deficit (Ferrio and Voltas 2005; Korol *et al.* 1999), among others. Although most of these works were limited to the last 100 or 200 years, such relationships have been recently extended over longer tree-ring chronologies, allowing high resolution climatic reconstructions back to *ca.* 11000 B.P. (see references in Heaton 1999). However, these studies are limited to a few world regions where such long-term chronologies are available. Even though they provide important keys for the understanding of global climate changes, it would be helpful to find alternative sources of information to refine climatic reconstructions at the local scale. In this context, Araus and Buxó (1993) proposed the use of $\delta^{13}C$ in charred grains from archaeological sites to get insight into the environmental conditions in early agriculture. This approach has been subsequently improved to allow the quantification of cereal water inputs (Araus *et al.* 1997a; Araus *et al.* 1999a) and crop yields (Araus *et al.* 1999b; Araus *et al.* 2003a). These works are based on the comparison with modern reference material, since $\delta^{13}C$ of seeds shows negligible changes during carbonisation, and thus the original isotopic signal is well preserved in charred remains (Araus *et al.* 1997b; Marino and DeNiro 1987). The impact of carbonisation on the $\delta^{13}C$ of wood has not yet been fully described, but indirect evidence shows that $\delta^{13}C$ in fossil charcoal also retains the original climatic signature of wood (February 2000; Van-Klinken *et al*. 1994; Vernet *et al*. 1996).

In this context, it is necessary to find some additional clues to interpret the inferences of ancient crop conditions derived from $\delta^{13}C$ analyses. Indeed, crop water availability, and thus $\delta^{13}C$ values in cereal crops, may be affected not only by climatic variations, but also by changes in crop management, such as irrigation practices (Araus *et al.* 1997b). To solve this question, one possibility is to compare $\delta^{13}C$ values found in cultivated plants with those from wild species (e.g. trees), which are not directly affected by agricultural practices. On the other hand, comparing the results from different crops can provide evidence about selective water management. In this work, we propose the use of combined $\delta^{13}C$ analyses of different plant species, as a way to get further information from isotope data, with the aim to discriminate between climate-derived and anthropogenic effects on crop water status, as well as to distinguish different strategies of water management.

## 4.2. Materials and Methods

### 4.2.1. Review on $\delta^{13}C$ data from archaeological grains

We compiled reported $\delta^{13}$C values from several archaeological sites in NE and SE Spain (Araus *et al.* 1997b; Araus and Buxó 1993) and in the Neolithic site of Tell Halula, in NW Syria (Araus *et al.* 1999a; Ferrio *et al.* 2003b). The dataset (see Table 4.1) included $\delta^{13}$C values from the cereals hulled barley (*Hordeum vulgare*), naked wheat (*Triticum durum*/*aestivum*) and the legumes faba bean (*Vicia faba minor*) and lentil (*Lens culinaris*).

### 4.2.2. Determination of $\Delta^{13}C$ values

To account for changes in $\delta^{13}$C of atmospheric CO$_2$ ($\delta^{13}C_{air}$) during the Holocene, we calculated plant $\Delta^{13}$C from $\delta^{13}C_{air}$ and plant carbon isotope composition ($\delta^{13}C_p$), as described by Farquhar *et al.* (1982):

**Eq. 4.1**

$$\Delta^{13}C_w = \frac{\delta^{13}C_{air} - \delta^{13}C_w}{(1 + \frac{\delta^{13}C_w}{1000})}$$

$\delta^{13}C_{air}$ was inferred by interpolating a range of data from ice-core records covering the whole Holocene (Eyer *et al.* 2004; Francey *et al.* 1999; Indermühle *et al.* 1999; Leuenberger *et al.* 1992). We recalculated $\Delta^{13}$C for all the archaeological data compiled, using these updated estimations of past $\delta^{13}C_{air}$.

### 4.2.3. Reference $\Delta^{13}C$ values in present crops

Average values of $\Delta^{13}$C in modern cereals and legumes were compiled from the literature (Araus *et al.* 1997b; Araus *et al.* 2003b; Ferrio *et al.* 2001; Voltas *et al.* 1999). The dataset included data from barley (*H. vulgare*), wheat (*T. aestivum* and *T. durum* combined) and faba bean (*V. faba*) grown in NE Spain, SE Spain and NW Syria, either under irrigated or rainfed conditions (see Table 4.2). We took advantage of the additional compiled data to update the model developed by Araus *et al.* (1999a) to estimate crop water inputs from $\Delta^{13}$C in wheat grains.

### 4.2.4. Meteorological data

In order to enable comparisons among data from different geographic origins, we used as reference values total precipitation from the second half of April to the end of May, coinciding, approximately, with the average grain filling period across the studied areas. Meteorological data was supplied by the *Instituto Nacional de Meteorología*, the *International Center for Agricultural Research in Dry Areas* (ICARDA) and the *Confederación Hidrográfica del Ebro*.

### 4.2.5. Statistical analysis

Heterogeneity of slopes ANOVA was performed to determine the differences between barley and wheat in their relationship between $\Delta^{13}$C and water inputs. $\delta^{13}C_{air}$ data from different sources was interpolated by fitting locally weighted least squares (LOESS) regression curves (Cleveland 1979). Due to the far greater time resolution of $\delta^{13}C_{air}$ data available since *ca.* 1800 A.D., we performed two separate LOESS regressions, one including data from 16100 cal. B.C. to 1800 A.D. (span = 0.1) and the other for the period 1798-1996 A.D. (span=0.4). The resulting curves were hereafter used as a single one, since they gave nearly identical estimations at the jointing point, around 1800 A.D. (end of the first curve: $\delta^{13}C_{air}(1800) = -6.402$; beginning of the second curve: $\delta^{13}C_{air}(1798) = -6.406$). A linear regression between $\Delta^{13}$C of archaeological grains of wheat and barley across sites were used to determine the degree of agreement between the two species, as well as to find potential outliers, according to the 95% confidence interval of the regression line.

**Table 4.1** Ages, chronological date, carbon isotope discrimination ($\Delta^{13}C$) and estimated water inputs (WI) during grain filling for the samples analysed. Mean plus standard error are indicated, as well as the number of samples, between parentheses. We included current average values of accumulated rainfall from the second half of April to the end of May (P ½ A+ M), as a reference for present WI during grain filling in the studied sites are. $\Delta^{13}C$ values recalculated from original $\delta^{13}C$ data reported by Araus and Buxó (1993), Araus *et al.* (1997b; 1999a) and Ferrio *et al.* (2003b), and updated estimations of air $\delta^{13}C$ (Eyer *et al.* 2004; Francey *et al.* 1999; Indermühle *et al.* 1999; Leuenberger *et al.* 1992).

| **Archaeological Site** | **Cultural period** | **Age (B.P. Cal)** | **Air $\delta^{13}C$ (‰)** | **$\Delta^{13}C$ (‰)** | | | | **WI (mm)** | | **P ½ A+M (mm)** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ***H. vulgare*** | ***T. aest/durum.*** | ***V. faba*** | ***L. culinaris*** | ***H.vulg.*** | ***T. aest/dur.*** | |
| *NE Spain* | | | | | | | | | | |
| L'Esquerda | Middle Ages | 700-800 | -6.4 | | 17.7 ± 0.4 (3) | | | | 143 ± 18 | 115 |
| St.Vicenç Enclar | Middle Ages | 800-900 | -6.4 | 15.9 ± 0.1 (2) | 17.0 (1) | | | 68 ± 3 | 108 | 105 |
| C.Mediona | Middle Ages | 800-900 | -6.4 | 16.9 ± 0.8 (2) | 16.4 (1) | | | 103 ± 32 | 88 | 88 |
| Empúries | Iron | 2300-2400 | -6.5 | 16.9 ± 1.2 (2) | 16.8 ± 0.2 (2) | | | 112 ± 48 | 103 ± 7 | 83 |
| Ullastret | Iron | 2300-2400 | -6.5 | 17.3 ± 0.6 (2) | 17.0 ± 0.3 (3) | | | 120 ± 25 | 110 ± 12 | 80 |
| Bòbila Madurell | Iron | 2400-2750 | -6.5 | 17.0 ± 0.0 (2) | 16.6 ± 0.2 (2) | | | 104 ± 1 | 94 ± 7 | 93 |
| Sitges UAB | Iron | 2550-2650 | -6.5 | 16.0 ± 0.8 (2) | 17.2 (1) | | | 75 ± 22 | 117 | 85 |
| Montou | Late Bronze | 2850-3350 | -6.5 | 17.6 ± 0.3 (2) | 17.0 ± 0.3 (2) | | | 132 ± 13 | 108 ± 10 | 83 |
| Montou | Middle Bronze | 3200-3700 | -6.5 | 17.3 (1) | 17.5 (1) | | | 116 | 131 | 83 |
| Institut de Manlleu | Chalcolithic/ Bronze | 4450-4850 | -6.3 | 17.3 ± 0.9 (2) | 17.2 (1) | | | 124 ± 40 | 116 | 115 |
| Cova 120 | Neolithic/ Chalcolithic | 4690-4910 | -6.3 | | 16.9 ± 0.0 (2) | | | | 104 ± 1 | 150 |
| Can Tintorer | Neolithic | 4690-5290 | -6.3 | 16.2 (1) | | | | 77 | | 75 |
| Montou | Neolithic | 5700-6290 | -6.4 | 17.9 (1) | 19.3 (1) | | | 143 | 251 | 83 |
| Plansallosa | Neolithic | 6710-6890 | -6.3 | 18.0 (1) | | | | 152 | | 143 |
| Cova 120 | Neolithic | 6600-7200 | -6.4 | 18.5 (1) | 17.4 ± 0.2 (2) | | | 181 | 127 ± 11 | 150 |
| La Draga | Neolithic | 7200-7750 | -6.6 | 17.5 (1) | 17.7 (1) | | | 125 | 140 | 135 |

**Table 4.1 (bis)**

| Archaeological Site | Cultural period | Age (B.P. Cal) | Air $\delta^{13}$C (‰) | $\Delta^{13}$C (‰) |  |  |  | WI (mm) |  | P ½ A+M (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | *H. vulgare* | *T. aest/durum.* | *V. faba* | *L. culinaris* | *H.vulg.* | *T. aest/dur.* |  |
| *SE Spain* |  |  |  |  |  |  |  |  |  |  |
| Fuente Amarga | Iron | 2300-2400 | -6.5 | 16.2 ± 0.8 (3) | 16.2 (1) |  |  | 84 ± 21 | 81 | 76 |
| Puente Tablas | Iron | 2400-2500 | -6.5 | 16.2 ± 0.4 (2) | 17.7 (1) |  |  | 79 ± 12 | 139 | 82 |
| Cuesta del Negro | Bronze | 3100-3190 | -6.5 | 18.0 (1) | 16.7 ± 0.6 (2) |  |  | 152 | 99 ± 20 | 53 |
| Los Palacios | Bronze | 3240-3520 | -6.5 | 15.3 ± 0.4 (3) | 15.0 ± 0.5 (3) |  |  | 57 ± 9 | 54 ± 8 | 75 |
| Peñalosa | Bronze | 3340-3570 | -6.5 | 18.1 ± 0.2 (2) | 18.5 (1) | 17.5 (1) |  | 154 ± 9 | 191 | 66 |
| Motilla del Azuer | Bronze | 3360-3720 | -6.4 | 16.4 (1) | 16.4 ± 0.7 (3) |  |  | 84 | 93 ± 25 | 75 |
| Motilla de las Cañas | Bronze | 3350-3750 | -6.4 | 17.2 (1) | 16.5 (1) |  |  | 113 | 92 | 75 |
| Castellón Alto | Bronze | 3520-3700 | -6.4 | 16.8 ± 0.2 (2) | 16.5 ± 0.1 (2) |  |  | 96 ± 7 | 91 ± 2 | 76 |
| Fuente Amarga | Bronze | 3530-3730 | -6.4 | 16.6 ± 0.9 (2) | 16.6 (1) | 15.4 (1) |  | 94 ± 30 | 96 | 76 |
| Cerro de la Virgen | Early Bronze | 3730-3840 | -6.4 |  | 16.3 ± 0.3 (2) | 18.5 (1) |  |  | 86 ± 9 | 76 |
| Las Pilas | Chalcolithic | 3840-3940 | -6.4 |  | 15.1 (1) | 19.1 (1) |  |  | 55 | 38 |
| Cerro de la Virgen | Campaniform | 3900-4040 | -6.4 | 18.2 (1) | 17.6 (1) | 19.1 (1) |  | 161 | 138 | 76 |
| Campos | Chalcolithic | 3925-4165 | -6.4 |  | 15.2 ± 0.6 (2) | 17.3 (1) |  |  | 57 ± 12 | 38 |
| Cerro de la Virgen | Pre-campaniform | 4000-4100 | -6.3 | 17.4 (1) | 17.1 (1) | 18.8 (1) |  | 120 | 112 | 76 |
| Los Millares | Chalcolithic | 3880-4240 | -6.4 | 17.3 ± 0.4 (2) | 17.5 (1) | 16.4 ± 1.2 (3) |  | 117 ± 16 | 132 | 42 |
| El Malagón | Chalcolithic | 3860-4265 | -6.4 | 15.5 (1) | 16.0 ± 0.5 (3) | 16.4 (1) |  | 59 | 78 ± 13 | 80 |
| Cueva del Toro | Late Neolithic | 5200-5700 | -6.4 |  | 16.2 (1) |  |  |  | 83 | 70 |
| Cueva del Toro | Late Neolithic | 5700-6500 | -6.3 |  | 16.5 (1) |  |  |  | 92 | 70 |
| Cueva del Toro | Middle/Late Neolithic | 5700-6500 | -6.3 | 17.3 ± 0.3 (2) | 16.8 ± 0.5 (4) | 17.1 ± 0.7 (2) |  | 116 ± 12 | 106 ± 15 | 70 |
| *NW Syria* |  |  |  |  |  |  |  |  |  |  |
| Tell Halula | Late Neolithic | 8400-9125 | -6.5 | 16.7 ± 0.4 (4) | 16.8 ± 0.5 (3) |  | 18.0 ± 0.5 (6) | 96 ± 14 | 106 ± 18 | 33 |
| Tell Halula | Late PPNB | 9125-9490 | -6.5 |  | 17.4 ± 0.3 (10) |  | 17.3 ± 0.4 (7) |  | 130 ± 13 | 33 |
| Tell Halula | Middle PPNB | 9600-9840 | -6.6 | 17.3 ± 0.2 (2) | 17.2 ± 0.3 (12) |  | 17.0 ± 0.3 (5) | 114 ± 10 | 123 ± 13 | 33 |

**Table 4.2** Average values of carbon isotope discrimination ($\Delta^{13}C$) in wheat, barley and faba bean grains grown under rainfed and irrigated conditions in the three geographic areas studied. Mean plus standard error are indicated, as well as the number of field trials analysed, between parentheses. For the rainfed trials, we include also water inputs (WI) during grain filling (estimated from $\Delta^{13}C$ values) and, for comparison, accumulated rainfall from the second half of April to the end of May (P ½ A+ M). $\Delta^{13}C$ values compiled from Araus *et al.* (1997b; 2003b), Ferrio *et al.* (2001) and Voltas *et al.* (1999).

| **Geographic area** | **Rainfed/ irrigated** | **$\Delta^{13}C$ (‰)** | | | **WI (mm)** | | **P ½ A+M (mm)** |
|---|---|---|---|---|---|---|---|
| | | ***H. vulgare*** | ***T. aest/durum*** | ***V. faba*** | ***H.vulgare*** | ***T. aest/durum*** | |
| NE Spain | Rainfed | 16.4 ± 0.5 (9) | 15.4 ± 0.9 (3) | | 82 ± 14 | 62 ± 20 | 70 |
| | Irrigated | 19.0 ± 0.4 (5) | 17.9 ± 0.5 (3) | | | | |
| SE Spain | Rainfed | 15.1 ± 0.5 (9) | 14.9 ± 1.0 (7) | 14.9 (1) | 51 ± 10 | 52 ± 15 | 59 |
| | Irrigated | 18.7 (1) | 16.9 ± 0.1 (6) | 16.7 ± 0.6 (3) | | | |
| NW Syria | Rainfed | 14.6 ± 0.6 (2) | 14.3 ± 0.5 (2) | | 42 ± 9 | 41 ± 8 | 35 |
| | Irrigated | | 16.8 ± 0.1 (2) | | | | |

## 4.3. Results and Discussion

### *4.3.1. Modelling water inputs from $\Delta^{13}C$ in grains*

The models relating $\Delta^{13}C$ in cereal grains and water inputs during grain filling are plotted in Fig. 4.1. (Araus *et al.* 1997a; Araus *et al.* 1999a) already reported strong positive relationships between water inputs and $\Delta^{13}C$ values from barley (*Hordeum vulgare*) and wheat (*Triticum durum*/*aestivum*) grains across a range of environmental conditions. After adding data from six wheat trials located at the Iberian Peninsula, both species provided nearly identical relationships, indicating that, despite their differences in growth cycle and ecological preferences, they show similar physiological responses to water availability. Nevertheless, wheat $\Delta^{13}C$ values tended to be smaller than those of barley, especially at the driest environments, in agreement with the greater sensitivity of wheat to water stress. On the other hand, it should be noted that, in both cases, the observed relationship was not lineal, and thus $\Delta^{13}C$ was more sensitive to water availability under harsher conditions. This can be explained by the fact that the main factor relating $\Delta^{13}C$ with water availability is the ratio between intercellular and atmospheric $CO_2$ concentrations, which is supposed to reach its maximum value in non-stressed plants. Thus, under near-optimum crop water status, no further increments in this variable, and thus on $\Delta^{13}C$, would be expected (Farquhar *et al.* 1989; Lambers *et al.* 1998).

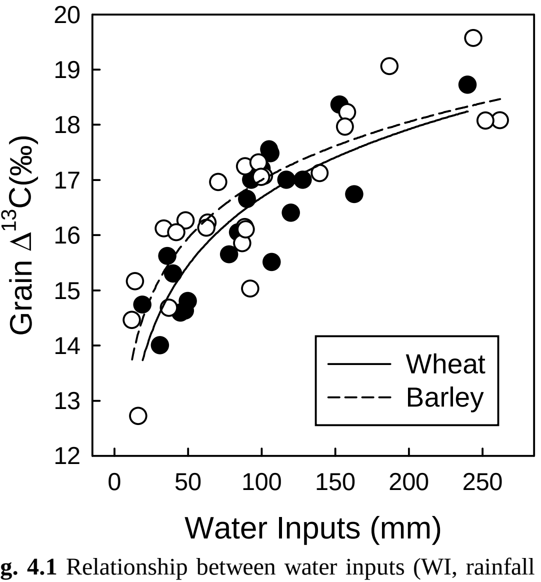

**Fig. 4.1** Relationship between water inputs (WI, rainfall plus irrigation if applied) during grain filling and $\Delta^{13}C$ of barley (*Hordeum vulgare*, empty circles) and wheat (*Triticum aestivum* and *T. durum*, filled circles) grains. Data from Araus *et al.* (1997a) for barley and from Araus *et al.* (1999a; 2003b) for wheat; $\Delta_{barley}$= 9.99 + 1.52*ln (WI), $r^2$=0.73, $P$<0.001, N=25; $\Delta_{wheat}$= 8.50 + 1.78*ln (WI), $r^2$=0.73, $P$<0.001, N=22.

### *4.3.2. Applications of $\Delta^{13}C$ to fossil plant remains*

### Reconstruction of past climate changes in the Mediterranean Basin

A case study of the application of archaeological plant remains to the analysis of climate fluctuations is shown in Fig. 4.2. The evolution of $\delta^{13}C_{air}$ during the Holocene, as inferred from ice-core records, is shown at the top of Fig. 4.2a. Average $\delta^{13}C$ values in cereal grains (wheat and barley combined) are plotted at the bottom of Fig. 4.2a. The data here presented was compiled from the archaeological sites described in Table 4.1, as well as from present agronomic trials (see Table 4.2). $\Delta^{13}C$ values were then recalculated from the updated estimations of $\delta^{13}C_{air}$ and measured $\delta^{13}C$ in grains, as described in equation 1 (Fig. 4.2b). Finally, by applying the modelled relationship between $\Delta^{13}C$ and water inputs (Fig. 4.1), it was possible to estimate water availability in the past from $\Delta^{13}C$ of ancient grain samples (Fig. 4.2c). We observed an important decrease in water availability from Neolithic to present-time in the three regions of study, specially clear for the two driest areas (SE Spain and NW Syria). These results indicate that, during early agriculture, cereals were cultivated under much better water status than that expected from present-day (rainfed) conditions in the same areas. This finding is in agreement with archaeobotanical evidence supporting the possibility that environmental conditions during early agriculture were cooler and moister than today, both in the Near East (Harlan 1998; Willcox 1996) and the Iberian Peninsula (Vernet 1990). The origin of such increase in aridity appears to be relatively recent, and has been probably enhanced by human activities. Indeed, several palaeoenvironmental records have evidenced a considerable decrease in precipitation in the Mediterranean since mid XIXth century, along with a global rise in temperature (Barriendos and Martín-Vide 1998; Creus *et al.* 1996; Folland *et al.* 2001; Riera *et al.* 2004).

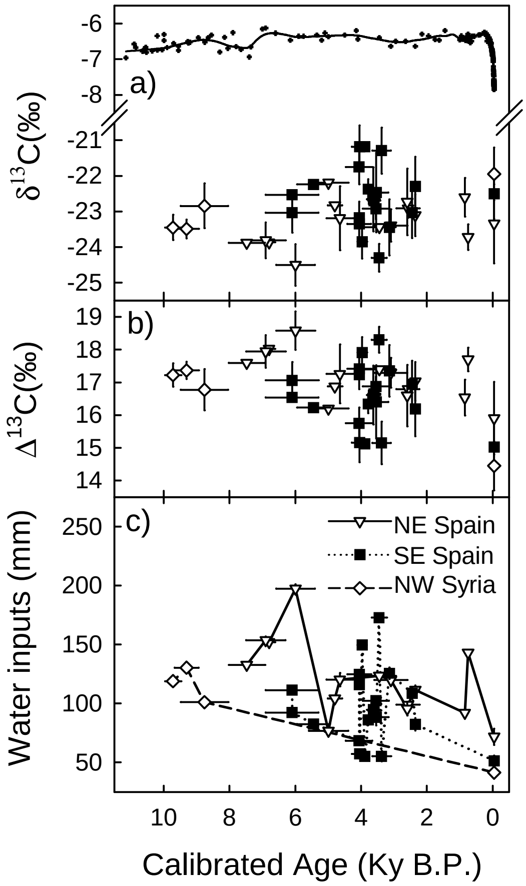

**Fig. 4.2** Estimation of past water inputs from carbon isotope discrimination ($\Delta^{13}C$) of archaeological cereal grains (*Hordeum vulgare* and *Triticum aestivum/durum* combined) collected from archaeological sites located in three areas of the Mediterranean Basin (NE Spain, SE Spain and NW Syria, see Table 4.1). (a) Evolution of the isotopic composition ($\delta^{13}C$) in atmospheric $CO_2$ (top) and archaeological cereal grains (bottom). Both variables (air and plant $\delta^{13}C$) are required to calculate $\Delta^{13}C$ of grains (b), as described in Equation 4.1. (c) Evolution of cereal water inputs (estimated from $\Delta^{13}C$) from Neolithic to present times in the areas studied.

### Is it feasible to discriminate between climatic and anthropogenic effects on $\Delta^{13}C$?

As cereals are cultivated plants, they may reflect not only climatic fluctuations, but also the effect on water status of certain agronomic practices, such as sowing in naturally wet soils (Hillman 1996) or irrigation (Helbaek 1960). This might be the case, for example, in some sites from NE and SE Spain, where estimated water inputs fell over the expected values for rainfed cultivation, even considering a wetter climate in the area (e.g. Montou and Cova 120, in NE Spain; Puente Tablas, Peñalosa, Cuesta del Negro, Cerro de la Virgen and Los Millares, in SE Spain). A possible way to discriminate between natural and anthropogenic changes in plant water status is to compare the carbon isotope signature of cultivated species with that of wild species, such as forest trees. This is exemplified in Fig. 4.3, where we compared the estimated water inputs for barley and wheat grains collected in NE Spain with $\Delta^{13}C$ values in wood charcoals from SE France, calculated from $\delta_a$ (see Fig. 4.2a) and wood $\delta^{13}C$ data reported by Vernet *et al.* (1996) for *Quercus humilis* (deciduous oak) and *Juniperus sp.* (juniper). As these two areas are close to each other and display similar climate regimes, we would expect to find common trends in both studies if climate were the main factor determining crop water status. As in cereals from NE Spain, Vernet *et al.* (1996) found evidence of higher water availability in the past than in the present-day. Moreover, both studies displayed relatively dry episodes (around 7000 B.P., 5000 B.P., 3000 B.P. and 1000 B.P.), alternating with more humid periods (around 6000 B.P. and 4000 B.P.), and concluding with a new decrease in water availability during the last millennium. The relatively good agreement between the results obtained for cereals and forest species suggests that most of the observed changes in crops may be explained by climatic conditions. As a way to further check the climatic effect on crop species, we are currently determining the carbon isotope signature of charcoals from the same sites where cereal grains were collected (Ferrio, Alonso, López, Araus and Voltas, submitted manuscript). By comparing the results of forest and cultivated species co-occurring in the same sites, it may be possible to estimate more accurately the relative influence of human activities on the water status of crops.

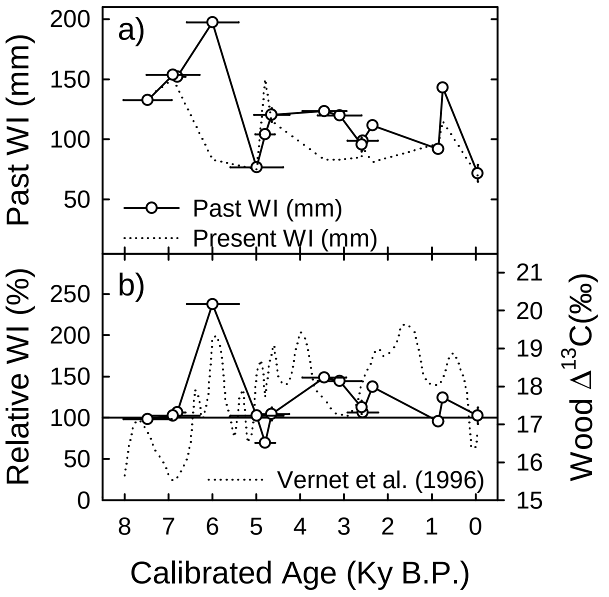

**Fig. 4.3** (a) Evolution of estimated water inputs (WI) in archaeological cereal grains (*Hordeum vulgare* and *Triticum aestivum/durum* combined) collected in archaeological sites from NE Spain, compared with average present values in the sites (as described in Table 4.1). (b) Evolution of WI, as a percentage respect current values. For reference, a horizontal line is plotted at WI=100% of present values. The dotted curve indicates the evolution of $\Delta^{13}C$ in wood charcoals of deciduous oak (*Quecus humilis*) and juniper (*Juniperus* sp.), collected at several archaeological sites from SE France. Charcoal $\Delta^{13}C$ values were calculated from wood $\delta^{13}C$ (Vernet *et al.* 1996) and air $\delta^{13}C$, as described in Equation 4.1.

An alternative approach to detect selective water management strategies is based on the comparison of crop water status among different crop species grown in the same area. This is shown in Fig. 4.4, putting together all the data described in Table 4.1. Hence, we compared the relationship across sites between $\Delta^{13}C$ of barley and wheat grains (Fig. 4.4a) and that between $\Delta^{13}C$ of cereals (barley and wheat combined) and either faba bean (*Vicia faba minor*) or lentil (*Lens culinaris*) (Fig. 4.4b). We found that $\Delta^{13}C$ values in barley and wheat grains were well correlated, showing similar trends across the sites. This suggests that both cereals were grown under similar conditions, and that observed fluctuations were mostly due to common (e.g. climatic) factors affecting their water status. In contrast, values of $\Delta^{13}C$ for faba beans and lentils were unrelated to those of cereal grains, being also significantly higher (about 1‰ in average). Differences in $\Delta^{13}C$ between cereals and grain legumes could be explained (at least in part) in terms of differences in grown pattern. Whereas cereals are determinate plants, producing all ears at one time, and grain growth coincides with the onset of drought in the Mediterranean region, legumes are indeterminate plants, producing successive pods throughout the crop cycle (generally from March to June). Therefore, for faba beans and lentils, most seeds may develop under a higher water status. On the other hand, the possibility that legumes were irrigated, even when cereals were cultivated under rainfed conditions, cannot be discarded. Indeed, we found that $\Delta^{13}C$ of archaeological faba beans were closer to those measured in present-day irrigated crops near the sites than to those of rainfed crops (see Table 4.2). On the other hand, the frequency of legume seeds found in the archaeological contexts studied was considerably lower than that of cereal grains. In spite of the uncertainties associated to the uneven

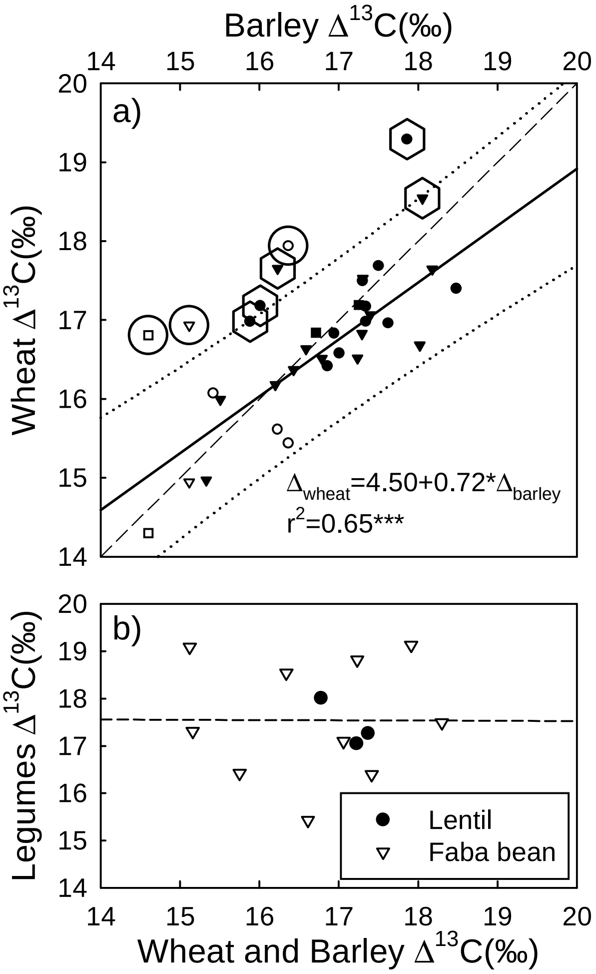

**Fig. 4.4** Relationship between $\Delta^{13}C$ values of hulled barley (*Hordeum vulgare*) and either naked wheat (*Triticum aestivum/durum*) or faba bean (*Vicia faba minor*) across all the archaeological sites from Table 4.1. (a) Detection of potential outliers for the relationship between wheat and barley, taking present data for comparison. Empty symbols, present-day samples, used for reference (see Table 2); filled symbols, archaeological samples; dotted lines, 95% confidence intervals for the regression line; dashed line, 1:1 reference line. Origin of the samples: circles, NE Spain; triangles, SE Spain; squares, NW Syria. The encircled points correspond to present-day values for irrigated wheat, against rainfed barley; the points surrounded by hexagons are "outlier" archaeological sites where wheat displayed higher $\Delta^{13}C$ values, probably indicating differential agronomic practices for wheat and barley. (b) Relationship between $\Delta^{13}C$ of legumes (faba bean and lentil) and cereals (wheat and barley combined), indicating the flat ($r^2$=0.00) regression line obtained (i.e. no relationship).

preservation of different types of plant remains (Wilson 1984; Wright 2003), the relative frequencies of cereals and legume crops might indicate that the area under grain legumes would be very small compared with that of cereals. If this were the case, such small, cultivated areas would be probably devoted to labour-intensive crops (i.e. garden crops) with supplementary irrigation, thus explaining the high $\Delta^{13}C$ values observed. Intensive (i.e. under irrigation) cultivation of faba bean could ensure a basic protein source in the human diet (Heiser 1990). The combination of extensive cereal crops expanding around human settlements with smaller plots of vegetables and legumes close to the living area has been reported to be the most common pattern in subsistence and pre-industrial agriculture (Hillman 1973). The above data suggest that this kind of management of water and soil resources could have been initiated during the early phases of agriculture.

Further analysis of the relationship between barley and wheat $\Delta^{13}C$ across the sites (Fig. 4.4a) can provide additional information. Despite both species followed the same relationship between water inputs and $\Delta^{13}C$ (see Fig. 4.1), $\Delta^{13}C$ values of wheat in archaeological sites were generally lower than those of barley (most points fell bellow the 1:1 reference line plotted in Fig. 4.4a). This suggests that barley grains were grown with greater water availability than wheat. Would this mean that barley was selectively grown under better conditions than wheat? Apparently not: if we consider the current growth cycles of the two species, we will find that barley grows up faster than wheat, reaching maturity about two weeks earlier. Thus, even growing at the same site, barley grains in Mediterranean climates are generally formed under moister conditions than wheat grains, as drought has a steep increase during the last weeks of the crop cycle (May-June). Consequently, it is expected barley grains show generally higher $\Delta^{13}C$ values than the co-occurring wheat grains. However, in Fig. 4.4a there are some points where wheat has a considerably greater $\Delta^{13}C$ than barley, falling far away from the fitted regression line (St. Vicenç Enclar, Sitges UAB and Montou, in NE Spain; Puente Tablas and Peñalosa, in SE Spain, see also Table 4.1). It is likely that these potential outliers indicate some selective treatments to enhance the performance of wheat crops, but not applied in barley, including irrigation or sowing in naturally wet alluvial soils (Araus *et al.* 1997b; Bar-Yosef *et al.* 1989). When we plotted in Fig. 4.4a present-day values of rainfed barley, compared with either rainfed or irrigated wheat, we found that the plots for rainfed wheat fitted well within the regression line for the archaeological sites. In contrast, the plots of rainfed barley related to irrigated wheat fell out of the 95% confidence prediction intervals of the regression line, and showed values for wheat $\Delta^{13}C$ similar to those of the presumed "archaeological“ outliers. This further supports the idea that sites showing greater wheat $\Delta^{13}C$ involved a differential water management for wheat and barley. As barley is less drought-sensitive than wheat, it is a common practice in dry areas to keep barley as a rainfed crop, reserving any additional water supply, or the moister soils (e.g. closer to a water stream) for wheat. Currently, this practice is enhanced by the fact that most barley is used for animal feeding, whereas wheat is preferred for human consumption. Again, we can find evidences of water and soil management practices during early agriculture that resemble those currently found in some areas. This could be of interest not only to reconstruct ancient agronomic techniques, but also to evaluate the potential long-term impact of current practices, looking at their consequences in the past.

## 4.4. Conclusions

Despite the drawbacks of inferring past conditions based on present relationships, this novel approach can help to identify events in which early farmers started to develop a conscious water management to improve their crops performance. It seems also possible to relate these events to the climatic conditions in which they were developed, or look for possible changes in response to climate and/or landscape modifications. In particular, our results suggest that, in general, ancient cereals (both wheat and barley) were grown under better water status than present-time crops. The more favourable water conditions were probably due to climatic variations, as suggested by the relatively good agreement between crop and forest plant remains. Nevertheless, we found evidences that, in some cases, wheat was probably favoured by ancient farmers, sowing it in better soils than barley, or under supplementary irrigation. Our results also indicate that grain legumes were usually grown under more humid conditions than cereals, most likely in small irrigated plots.

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# Chapter 5

*"…y aunque en rigor no es mejor,*
*por ser mayor o menor,*
*una encuesta hice a mi alrededor."*

Javier Krahe. *Un burdo rumor*

## Chapter 5

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# Estimating grain weight in archaeological cereal crops: a quantitative approach for comparison with current conditions

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**Abstract** Data relating to grain weight of cereal crops cultivated in the past could be useful to track early genetic and/or agronomic improvements, facilitating the comparison of archaeological data with current agronomic studies. However, archaeological grains are usually preserved by a process of carbonisation, during which there is a considerable reduction in mass and dimension changes. The aim of this study was to develop a model for the estimation of original grain weight from the dimensions of charred grains, taking into account the effect of carbonisation. For this purpose, extant grains of wheat and barley were experimentally carbonised at three temperature levels (200ºC, 250ºC, 300ºC) and under two atmospheric conditions (oxidant, reducing). Weight, length (L), breadth (B) and thickness (T) were measured before and after carbonisation. The products L×B and L×T provided the best estimation of grain weight ($r^2$=0.80-0.86) and were relatively stable across treatments. As a case study, we applied grain weight models to wheat and barley grains gathered from a range of archaeological sites in the Segre and Cinca Valley (Catalonia, NE Spain, ca. 3900-2200 cal. BP). In this region, we observed a relatively constant increment in grain weight during the first half of I millennium B.C.E.; probably explained by the increased water availability indicated by palaeoenvironmental studies. In contrast, the increased grain weight of current samples, compared with that of archaeological grains, is largely attributable to more recent genetic improvements.

**Keywords** agriculture • charred seeds • dimensions • morphology • carbonisation • wheat • barley

## 5.1. Introduction

Grain weight is one of the three main agronomic components of grain yield in cereals, along with the number of spikes per unit grown area and the number of grains per spike; moreover, it has direct implications for grain quality (Molina-Cano et al. 1997; Evers et al. 1995). Therefore, data relating to grain weight of cereal crops cultivated in the past would be of interest as a way to track genetic improvement in this trait, but also as an indicator of the potential quality of the food products that could be delivered from them. Although strongly genetically determined, grain weight also depends on environmental constraints, such as water availability or temperature stress (Voltas et al. 1999b; Rharrabti et al. 2003). For example, when crops are initially grown under favourable conditions, but become stressed during

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grain filling (which is a common case in rainfed Mediterranean environments), the time span for grain growth is reduced and grain weight is limited (Gooding et al. 2003; Savin and Molina-Cano 2002). Thus, estimates of grain weight in ancient cereal crops could also provide helpful information about the agronomic conditions under which the plants were grown.

From an archaeological perspective, current excavation techniques often involve the systematic recovery of carbonised plant remains, including grains from cereals and other crops, and grain dimensions are routinely measured. However, grain proportions change considerably after charring and, consequently, are not directly comparable with those of intact grains (Hopf 1955; Lone et al. 1993). Similarly, although archaeological grains can be weighed, their weight is not directly comparable with the original weight, as there can be considerable loss during carbonisation (Threadgold and Brown 2003). Moreover, grains are often found partially broken and/or with mineral inclusions, still allowing reliable measurements of principal dimensions, but not of the total weight. In contrast, current agronomic studies are conducted with non-carbonised grains and, whereas grain dimensions are rarely considered, grain weight is measured routinely (Cascón 1934; Henry and Kettlewell 1996; Savin and Molina-Cano 2002). Consequently, being able to estimate grain weight in archaeological grains would allow us to compare them with the large body of modern agronomic data currently available. Hence, the aim of this work was to find a way to link archaeobotanic and agronomic studies, as the comparison between such extensive sources of data might help to answer some unsolved questions. Our specific objective was to develop a model to estimate original grain weight from charred grain dimensions that would be applicable to archaeological cereal seeds. We analysed the effect of carbonisation on principal grain dimensions (length, breadth and thickness), morphological indices and grain weight. A wide range of carbonisation levels, from slightly toasted to fully carbonised or nearly combusted, under either aerobic or anaerobic conditions, was assayed. By including such a range of charring conditions in model calibration, we expected to cover the variability found in archaeological assemblages. A case study on archaeological seeds recovered from several sites in the Segre and Cinca Valley (Catalonia, NE Spain) is presented to illustrate the application of these models in an archaeological context.

## 5.2. Experimental procedure

### *5.2.1. Plant material and charring conditions*

The relationship between charred grain dimensions and grain weight was modelled using extant grains of barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.), harvested in the experimental fields of UdL-IRTA centre in Lleida (Catalonia, NE Spain). We took 144 grains for each species, covering a wide range of grain weight, dimension and morphology (Table 5.1).

We assigned grains to six separate subsets, selected to include a similar range of grain weight and dimensions. We ensured that there were no significant differences in grain weight and dimensions among the subsets. All samples were dried at 60ºC for 24 hours, and submitted to a pre-heating ramp of two hours to minimise grain explosion (i.e. ‘pop-corn’ effect) due to violent output of water and combustible gases. The subsets were carbonised under three levels of maximum temperature (200ºC, 250ºC, 300ºC) and two atmospheric conditions (oxidant, reducing). Samples were kept at these temperatures for 45 min. To obtain a reducing atmosphere, three of the subsets were buried in high crucibles under 3 cm of sand, whereas the other three (oxidant) were placed in flat crucibles without burying.

### *5.2.2. Grain measurements*

Weight and dimensions were determined before and after the experimental carbonisation of extant grains. We measured length (L), breadth (B) and thickness (T) under a stereoscopic microscope with an LCD camera coupled to a PC, assisted by image analysis software (Leika Qwin Lite 2.1, 1997). After carbonisation, some grains were too distorted and/or broken to be measured and were not included in the analysis.

**Table 5.1** Mean and range for grain weight, dimensions and morphological indices before and after experimental carbonisation. The data shown here correspond to global values, i.e. across the six treatments assayed. N, number of samples.

| **Grain Variable** | **Intact grains** | | **Charred grains** | |
|---|---|---|---|---|
| | **Range** | **Mean** | **Range** | **Mean** |
| *Wheat* | *N=144* | | *N=95* | |
| Grain weight (mg) | 7.3 - 61.4 | 29.5 | 3.5 - 55.0 | 20.9 |
| Length (L, mm) | 4.1 - 7.4 | 5.7 | 3.4 - 7.1 | 5.1 |
| Breadth (B, mm) | 1.5 - 3.6 | 2.6 | 1.9 - 5.4 | 3.3 |
| Thickness (T, mm) | 1.4 - 3.4 | 2.4 | 1.6 - 4.8 | 2.7 |
| L/B | 1.8 - 3.7 | 2.2 | 0.9 - 2.8 | 1.5 |
| L/T | 1.9 - 4.5 | 2.4 | 1.0 - 3.4 | 1.8 |
| B/T | 0.7 - 1.7 | 1.1 | 1.0 - 1.5 | 1.2 |
| *Barley* | *N=144* | | *N=92* | |
| Grain weight (mg) | 3.4 - 56.5 | 28.0 | 3.4 – 48.5 | 20.0 |
| Length (L, mm) | 3.8 - 7.7 | 6.3 | 3.8 - 7.9 | 6.1 |
| Breadth (B, mm) | 1.6 - 3.7 | 2.9 | 1.9 - 4.7 | 3.3 |
| Thickness (T, mm) | 0.7 - 3.0 | 2.1 | 1.1 - 4.1 | 2.6 |
| L/B | 1.7 - 3.0 | 2.2 | 1.2 - 2.7 | 1.9 |
| L/T | 2.2 - 7.4 | 3.1 | 1.6 - 4.8 | 2.5 |
| B/T | 1.2 - 2.5 | 1.4 | 1.0 - 2.1 | 1.3 |

### *5.2.3. Statistical analyses*

Data were subjected to analysis of variance (ANOVA) to determine the effect of carbonisation treatments (temperature and atmospheric conditions). Linear regressions were calculated to assess the relationships between original grain weight and dimension variables. Unless otherwise stated, differences were considered statistically significant when P<0.05. All analyses were carried out using standard SAS-STAT procedures (SAS 1988).

Model fitness was assessed using root mean square error (RMSE):

**Eq. 5.1**

$$RMSE = \sqrt{\frac{\sum (Y_{ref} - Y_{pred})^2}{(n-p)}}$$

where $Y_{ref}$ and $Y_{pred}$ are observed and predicted values for each sample, $n$ stands for the number of samples and $p$ is the number of estimated parameters ($p$=2). From this value we could obtain the standard error ($SE_p$) for each predicted value, which is a function of all errors associated with the regression equation (Bowley 1999):

**Eq. 5.2**

$$SE_p = RMSE * \sqrt{\frac{(X - X_m)^2}{\sum X^2} + \frac{1}{n}}$$

where *RMSE* and $n$ are as described above, $X$ is the value of the independent variable for a given sample and $X_m$ stands for the mean value of $X$ over all samples.

Grain weight of archaeological seeds was estimated from the products Length x Breadth (L×B) and Length x Thickness (L×T), taking the mean value of both estimates. RMSE could not be calculated for archaeological samples, as $Y_{ref}$ (i.e. real values) were unknown. Instead, we took root mean square difference (RMSD) between the values estimated from the two variables (L×B and L×T), as a rough indicator of the prediction error in archaeological samples:

**Eq. 5.3**

$$RMSD = \sqrt{\frac{\sum (Y_{LB} - Y_{LT})^2}{n}}$$

where $Y_{LB}$ and $Y_{LT}$ stand for the predicted values using either the L×B or L×T model, and $n$ is the number of samples. RMSD was also calculated within calibration samples as a reference value for archaeological results.

The overall standard error for a given archaeological sample ($SE_{sample}$) and site ($SE_{site}$) was calculated following the general rules of error propagation (Ku 1966):

**Eq. 5.4**

$$SE_{sample} = \sqrt{\frac{\sqrt{(SE_{pLB})^2 + (SE_{pLT})^2}}{2} + (SE_{\overline{LB,LT}})^2}$$

**Eq. 5.5**

$$SE_{site} = \frac{\sqrt{\sum (SE_{sample})^2}}{N}$$

where $SE_{pLB}$ and $SE_{pLT}$ are the $SE_p$ associated with the estimation of grain weight from L×B and L×T, respectively, $SE_{\overline{LB,LT}}$ represents the standard error of the mean between the two estimates (from L×B and L×T) and $N$ stands for the number of samples within each site.

## 5.3. Results and discussion

### *5.3.1. Changes in grain size and morphology during carbonisation*

Overall, both grain weight and grain dimensions changed considerably after charring, thus confirming that they are not directly comparable with those of intact seeds (Table 5.1). We observed an average depletion in grain weight of about 30%, and the grains became more rounded, especially in wheat. There was a general decrease in L (11% in wheat, 5% in barley), whereas B and T increased (for B: 27% in wheat, 14% in barley; for T: 13% in wheat and 23% in barley). Similar changes in grain dimensions during carbonisation have been reported previously for several species of wheat and barley, under a variety of carbonisation conditions (Hopf 1955; Lone et al. 1993; Willcox 2004; Renfrew 1973; Colledge 1994). In all cases, charred grains tended to shrink in length, whilst increasing in breadth and thickness. In spite of these general trends, the quantitative effect of charring varied considerably according to the species and experimental conditions. This is partly confirmed by our work, as grain weight and dimensions after charring differed significantly between temperatures (Table 5.2). However, we did not find any significant effect of atmospheric conditions on the variables studied.

Comparing the two species, we observed that the degree of distortion was somewhat greater in wheat than in barley. Probably, the greater density (i.e. tighter packing) of wheat (75-80kg/hl) than

**Table 5.2** Effect of carbonisation conditions on weight and grain dimensions, and determination coefficients (r$^2$) of the linear relationship (across treatments) between initial grain weight and different variables measured after carbonisation. Mean values for intact grains (i.e. before carbonisation) are included for reference (N=144). Significance of the effect of temperature, atmosphere and their interaction is indicated for each variable (* $p$<0.05, ** $p$<0.01, *** $p$<0.001). Mean values with the same letter are non-significantly different according to the LSD test. Linear regressions are all significant at $p$<0.001. Measurable samples (%), percentage of the original samples still measurable after carbonisation; L, length; B, breadth; T, thickness.

| **Treatment** | **Measurable samples (%)** | **Grain weight (mg)** | **L (mm)** | **B (mm)** | **T (mm)** | **L*B*T (mm$^3$)** | **B*T (mm$^2$)** | **L*B (mm$^2$)** | **L*T (mm$^2$)** |
|---|---|---|---|---|---|---|---|---|---|
| Intact grains | | | | | | | | | |
| *Wheat* | | 29.5 | 5.7 | 2.6 | 2.4 | 36 | 06.2 | 14.8 | 13.7 |
| *Barley* | | 28.0 | 6.3 | 2.9 | 2.1 | 38 | 06.1 | 18.3 | 13.2 |
| Charred grains | | | | | | | | | |
| *Wheat* | | | | | | | | | |
| Temperature | | *** | *** | n.s. | * | n.s. | * | n.s. | n.s. |
| Atmosphere | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Temp. × Atm. | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean values | | | | | | | | | |
| 200ºC | 100 | 27.7 a | 5.5 a | 3.3 a | 2.6 a | 48 a | 08.6 a | 18.2 a | 14.6 a |
| 250ºC | 69 | 15.4 b | 4.6 b | 3.4 a | 2.9 ab | 47 a | 10.0 ab | 16.0 a | 13.4 a |
| 300ºC | 29 | 10.5 b | 4.6 b | 3.5 a | 2.9 b | 50 a | 10.5 b | 16.0 a | 13.7 a |
| r$^2$ (across treatments) | | 0.67 | 0.43 | 0.50 | 0.49 | 0.77 | 0.52 | 0.80 | 0.82 |
| *Barley* | | | | | | | | | |
| Temperature | | *** | * | ** | *** | ** | *** | n.s. | n.s. |
| Atmosphere | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Temp. × Atm. | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean values | | | | | | | | | |
| 200ºC | 100 | 25.3 a | 6.3 a | 3.2 a | 2.4 a | 51 a | 07.8 a | 20.5 a | 15.2 a |
| 250ºC | 73 | 14.8 b | 5.8 b | 3.4 a | 2.8 b | 56 ab | 09.5 b | 19.8 a | 15.9 a |
| 300ºC | 19 | 12.2 b | 6.3 ab | 3.8 b | 3.3 c | 85 b | 12.8 c | 24.7 a | 21.0 a |
| r$^2$ (across treatments) | | 0.69 | 0.64 | 0.67 | 0.61 | 0.77 | 0.65 | 0.84 | 0.82 |

barley grains (60-65kg/hl) enhanced the distortion during charring. Nevertheless, despite showing greater deformations, they were less dependent on the carbonisation environment for wheat than for barley (Table 5.2). In wheat, L was the most variable dimension, whereas for barley T was the least consistent. As our aim was to develop a model sufficiently robust to be applied over a wide range of seed morphologies, the best variables would be those that are relatively stable regardless of carbonisation conditions. The products L×B and L×T were the only two variables not showing significant differences among treatments in both species. Apparently, the increment in either B or T brought about by higher temperatures was compensated by the reduction in L, thus giving a more stable variable than each dimension taken separately. In addition, L×B and L×T showed the strongest relationships with grain weight across all treatments (Table 5.2). Consequently, the variables of choice for model calibration were L×B and L×T. As expected, although the weight of charred grains showed a relatively good correlation with original grain weight across treatments, it was highly dependent on the charring conditions. Indeed, grain weight showed little variation when carbonisation was performed at 200ºC (the lowest temperature assayed), but was reduced drastically (about 50%) at temperatures above 250ºC (Table 5.2). This, added to the aforementioned considerations (grain fragmentation, presence of mineral inclusions and other taphonomic alterations), confirms that this variable cannot be used as an estimator of original weight in archaeological cereal seeds.

### *5.3.2. Sample preservation: does initial grain weight affect sample survival during charring?*

As the degree of carbonisation increased, a greater number of samples were either destroyed or too distorted to be measured (Table 5.2). In order to account for potential drifts due to sample loss, we checked whether or not the "surviving" seeds differed in their original grain weight when compared with the rest of the samples. There were no significant differences (data not shown) and, consequently, no evidence of a differential preservation of seeds related to their initial grain weight. Moreover, we did not find any significant relationship between original grain weight and the changes in grain dimensions during carbonisation. This suggests that grain size of a carbonised cereal sample is likely to be representative of the original sample, provided that there is no further drift during burial and recovery. Wilson (1984) and, more recently, Wright (2003), assayed the effect of different carbonisation environments on the relative survivability of weed seeds, both concluding that it was strongly dependent on the heating conditions. In a series of field experiments, including experimental fires, sampling and flotation, Gustafsson (2000) also found that weed seeds showed contrasting levels of carbonisation, even under the same conditions, and suggested that this could be related to substantial differences in seed composition (e.g. oil content). However, in the same experiment, grains from different cereal species were carbonised and recovered in similar proportions, probably due to their relatively uniform chemical composition. Considering the great morphological variability found among cereal species, the work of Gustafsson supports the absence of any differential preservation of cereal grains according to their size and/or morphology. Nevertheless, random drifts should not be discounted, although they can be minimised by increasing sample size, as well as including samples from several carbonisation events (Gustafsson 2000; Boardman and Jones 1990; Buxó 1997).

### *5.3.3. Grain weight models: performance and range of application*

Once the most suitable variables were selected, we used regression models to predict grain weight, pooling samples from all treatments together, with the aim of covering the widest range of experimental situations. In both species, L×B and

L×T were strongly related to grain size (r2=0.80-0.86), although they showed different trends in wheat and barley (Fig. 5.1). In wheat, both L×B and L×T were linearly related to grain size. However, in barley the relationship between these variables and grain size was improved using a power model (Fig. 5.1c, d). This could be explained by the lower morphological distortion of barley grains, as the relationship between these variables and weight in intact grains of both species fitted power curves (data not shown). RMSE values of 5.1-5.4 mg (Fig. 5.1) suggest a good predictive accuracy of the models. Nevertheless, taking the mean between the values estimated from L×B and L×T gave the most reliable estimations of grain weight, and RMSE was lower (4.9 mg and 5.0 mg, for wheat and barley, respectively) than for each of them taken separately. Within the whole calibration range, SEp was always less than 2 mg, and it was less than 1 mg in the most common ranges of 9-44 mg and 10-46 mg of predicted values, for wheat and barley, respectively. It should be noted that the range of dimensions and morphology included in calibration was similar to that usually reported in archaeological grains for both species, which can be 3.0-7.5 mm in L, 1.7-4.6 mm in B and 1.5-4.0 mm in T (Willcox 2004; Colledge 2001; Buxó 1997; Lone et al. 1993; Alonso 1999; Alonso 1986; Alonso et al. 2002). Moreover, mean site

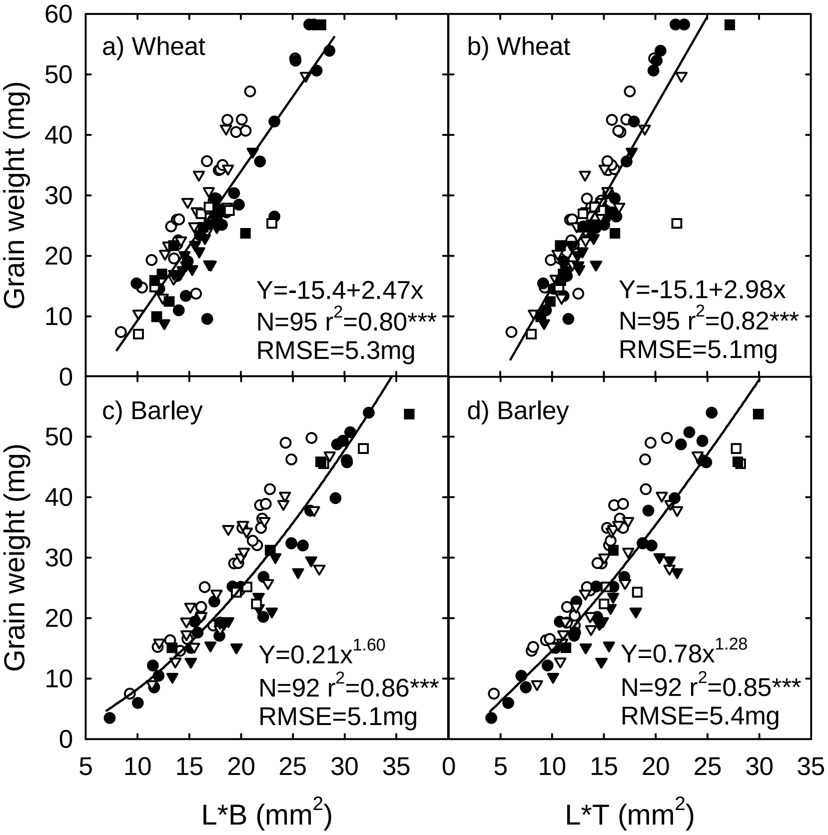

**Fig. 5.1** Regression models across treatments to estimate initial grain weight of wheat (a, b) and barley (c, d) from the products Length x Breadth (L×B; a, c) and Length x Thickness (L×T; b, d) of carbonised grains. Circles, triangles and squares indicate temperature levels of 200ºC, 250ºC and 300ºC, respectively. Open and closed symbols indicate reduction and oxidation treatments, respectively. All regressions were significant at *p*<0.001.

values reported in the literature would always be included within the aforementioned optimal range (SEp<1 mg). This suggests that current models can be applied to most archaeological cereal seeds, provided that they are sufficiently well preserved to allow reliable measurements.

## 5.4. A case study with archaeological samples

To further assess the suitability of the models, we applied them to a set of archaeological samples. Charred grains of naked wheat (Triticum aestivum/durum) and hulled barley (Hordeum vulgare) were gathered from six archaeological sites in the Segre and Cinca Valley (Catalonia, NE Spain, see Table 5.3), ranging from Bronze Age (ca. 3900 cal. BP) to Second Iron Age (Late Iberian period: ca. 2200 cal. BP). Currently, all these sites show similar climatic and soil conditions, and are characterised by a semiarid Mediterranean climate. The samples were gathered from various archaeological contexts, such as domestic fires, cooking ovens, storage jars, room floors and levels of rubble from housing structures and pits (for further details about the sites and sampling procedures, see Alonso 1999; Alonso 1986; Alonso et al. 2002). We determined grain dimensions in these samples as described in section 5.2.2. Seeds too distorted and/or broken were not measured.

Firstly, we confirmed that the range of values within the set of archaeological samples was suitable for the application of the models. Thus, 96% of the samples fell within the calibration range for grain dimensions and morphological indices described in Table 5.1, with about 95% of the samples falling within the optimal range (SEp<1 mg) of the models. Removing the samples that fell outside of these limits did not significantly affect the results, so we decided to keep them in the analysis. Moreover, calculated RMSD for archaeological samples (3.9 mg and 3.4 mg, for wheat and barley, respectively) was similar to that found within calibration samples (3.6 mg for both species), suggesting that the accuracy of the estimates in archaeological seeds was close to those reported for current samples (section 5.3.3).

**Table 5.3** Site description and number of archaeological grains used in the case study. Calibrated age represents the approximate age in years BP estimated from a combination of archaeological and calibrated $^{14}$C dating using the program CALIB 3. 03 (Stuiver and Reimer 1993).

| Site | Lat. | Long. | Cultural Period | Calibrated Age (BP) | Samples | |
|---|---|---|---|---|---|---|
| | | | | | Wheat | Barley |
| Minferri | 41º30’ | 0º45’ | Bronze Age | 4100-3650 | 118 | 29 |
| El Vilot III | 41º38’ | 0º30’ | Bronze Age | 3113-2794 | 13 | 17 |
| El Vilot II | 41º38’ | 0º30’ | Bronze Age | 2923-2393 | 14 | 70 |
| Els Vilars II | 41º34’ | 0º56’ | First Iron Age | 2550-2425 | 92 | 170 |
| Margalef | 41º34’ | 0º47’ | Second Iron Age | 2250-2150 | 100 | 100 |
| Tossal Tenalles | 41º37’ | 0º47’ | Second Iron Age | 2250-2150 | 100 | 100 |

Fig. 3.2 plots average grain weight for the six archaeological sites, taking the mean between the estimates from L´B and L´T models, as described in section 5.2.3. We also included a reference value for present-day samples of wheat (Araus et al. 2003; A.E.T.C. 2003) and barley (Voltas et al. 1997; Voltas et al. 1999a) grown in rain-fed conditions in the same area. We observed a large increase in grain weight of present-day samples as compared with that of archaeological grains. Indeed, even under the harsher conditions in which barley and wheat can grow, values of grain weight are usually over 30 mg, and rarely fall below 25 mg (Gooding et al. 2003; Acevedo et al. 1991; Araus et al. 1998). Such results contrast with the estimated grain size among the archaeological sites studied, which were always below 25 mg. Moreover, when we applied our models to other archaeological data acquired from the literature (Buxó 1997; Lone et al. 1993), estimated grain weight was never greater than 30 mg. Data from

geomorphology (Gutiérrez and Peña 1992), antracology (Ros 1997; Alonso et al. 2002) and palynology (Alonso et al. 2002) indicate that the current climate in the area is more arid than in the period studied. In addition, the analysis of carbon isotope composition of barley and wheat grains recovered from other archaeological sites in Catalonia also suggested that they were grown under much wetter conditions between the Neolithic period and the Middle Ages than currently (Araus and Buxó 1993). Thus, such a large increase in grain weight from archaeological to present times is mostly attributable to more recent advances in plant breeding and agronomic practices, including the extensive application of nitrogen fertilisers (Rharrabti et al. 2003; Acevedo et al. 1991; Gooding et al. 2003; Voltas et al. 1998). The differences observed among archaeological samples, however, appear to be related to environmental changes in the studied area. At the second half of the II millennium B.C.E., vegetation was characterised by evergreen, schlerophyllous species, forming associations of Rhamno-Quercetum cocciferae and Rosmarino-Ericion, typically Mediterranean (Ros 1997; Alonso et al. 2002). During the first half of the I millennium B.C.E., however, there was a progressive change in vegetation, with the formation of meso/submediterranean forests, composed of a mixture of oaks (evergreen and deciduous) and pines, together with shrub woodlands in the driest places (Ros 1997; Alonso et al. 2002). This is in agreement with the existence of a period of wetter climate, according to geomorphological studies (Gutiérrez and Peña 1992). Consequently, the increase in grain weight observed from 3000 BP to 2000 BP, particularly apparent in barley, may be due to changes in water availability. Nevertheless, we cannot rule out the possibility of genetic improvement in grain size (possibly unintentional in agronomic terms) during the period studied, as increased grain size implies

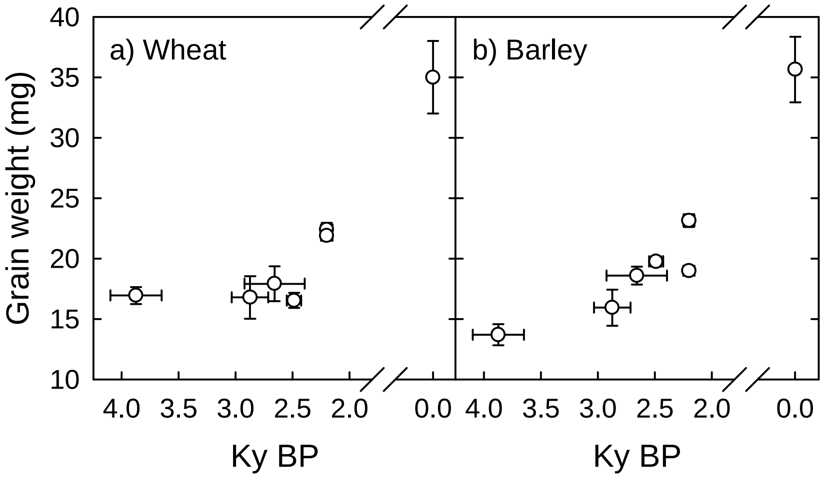

**Fig. 5.2** Evolution of grain weight of barley and wheat in the Segre and Cinca Valley (Catalonia, NE Spain) from Bronze Age (*ca*. 3900 cal. BP) to Second Iron Age (*ca*. 2200 cal. BP), compared with present-day samples (see Table 3 for a description of the archaeological sites). Archaeological values were estimated from the products Length x Breadth (L×B) and Length x Thickness (L×T), taking the mean value of both estimates. Present-day data correspond to barley (Voltas et al. 1997; Voltas et al. 1999) and wheat (A.E.T.C. 2003; Araus et al. 2003) grown under rain-fed conditions around the studied area. Horizontal error bars indicate the range of calibrated age. Vertical error bars of the archaeological samples indicate the standard error of predicted means, including within-site variability, regression error and the difference between predicted values using the two models (see text for details). For present-day data, vertical error bars indicate the standard error of the mean across environments (N=6).

better end products. Indeed, grain weight is directly associated with flour yield (Henry and Kettlewell 1996) and baking properties of wheat (Evers et al. 1995), and also determines malting quality of barley (Molina-Cano et al. 1997). Moreover, the differences between archaeological and current data were not limited to modern cultivars, but also applicable to traditional landraces (Acevedo et al. 1991; Araus et al. 1998; Cascón 1934), supporting the idea that genetic improvement in grain size had already started before the implementation of modern breeding programs. Indeed, grain size is an easily identifiable trait, and, for example, it has been documented that Spanish farmers have traditionally reserved the best seeds (i.e. the bigger) for sowing (Cascón 1934): it is probable that such practice began during the early phases of agriculture.

Nevertheless, we should bear in mind that carbonised plant remains are not necessarily a random sub-sample of the overall harvest. Indeed, carbonised grains might have been those discarded by the farmers, and thrown onto fires along with other plant debris (Dennell 1976; Boardman and Jones 1990). Exceptionally, for two of the sites studied (*Margalef* and *Tossal de les Tenalles*), there was evidence that the samples analysed were representative of harvested grains, as thousands of them were found within storage jars. For the remaining sites, however, we are unaware of the origin of the grains and, although a large number of samples (and carbonisation events) would reduce any biasing, these results should be interpreted with caution. Nevertheless, the difference in grain weight between stored grains and those recovered from other sources was much smaller than that between archaeological and present-day grains. Therefore, even considering only stored material (i.e. undoubtedly representative of harvested grains), we can conclude that a significant genetic improvement in grain weight occurred in the area, at least during the last two millennia.

## 5.5. Conclusions

We have confirmed that although there is considerable change in dimensions and morphology of wheat and barley grains during carbonisation, some measurements are relatively constant over a wide range of carbonisation levels. The products L×B and L×T were the best correlated with grain weight, and also the most stable across carbonisation treatments. The range of dimensions and morphology present in the sample set used for calibrations suggested that it was representative of the variety of (unknown) conditions in which archaeological grains were carbonised. Indeed, this range was wide enough to allow accurate predictions in about 95% of the archaeological seeds in which the models were tested. In our case study, we concluded that the increase in grain weight between archaeological samples and present-day crops was principally due to genetic improvements. Differences among archaeological samples, however, were apparently related to environmental change; although genetic differences could not be dismissed. Further work will be necessary to clarify the origin of such variations, as well as to determine the timing of the change in grain weight to its current values.

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# Chapter 6

*“Supongamos ahora que sembráis trigo candeal [...] el trigo crecerá de una manera uniforme [...] si existe alguna diferencia entre las espigas, se deberá a que habrán recibido distintos cuidados del labrador o a que habrán participado desigualmente de los beneficios de la lluvia y el rocío [...]”*

Kung Tsé. *Meng Tsé*

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# Evolution of grain weight in archaeological cereal seeds: potential role of climate and genetic improvement

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**Abstract** Inferences on grain weight (GW) of cereal crops cultivated in the past could be useful to track early agronomic improvements derived from empirical breeding. Though strongly genetically determined, GW also depends on environmental constraints, such as water availability during grain growth. In the archaeological context, it is possible to estimate GW from grain dimensions, while plant water status can be determined by the study of carbon isotope discrimination ($\Delta^{13}C$). The aim of this work was to determine the relative effect of environmental changes on the evolution of GW of naked wheat (*Triticum aestivum/durum*) and hulled barley (*Hordeum vulgare*) in the Segre and Cinca Valley (Ebro Depression, NE Spain) during the last four millennia. We performed grain measurements and $\delta^{13}C$ analyses in a set of charred grains, recovered from nine archaeological sites, spanning from Bronze Age (*ca*. 1950 BCE) to Middle Age (XI c. CE). Estimated GW in archaeological grains (19.6 mg in average for both species) was significantly smaller than the values found in present rainfed cereals cultivated in the area (32.5 mg). In contrast, average $\Delta^{13}C$ values did not differ significantly (15.93‰ and 15.90‰, for archaeological and modern material, respectively. Accordingly, the most recent increase in GW cannot be attributed to increased water availability. We concluded that the greater grain weight in present-day cereals responded to a genetic improvement of the crops, which might have started during the Middle Age.

**Keywords** agriculture • charred seeds • dimensions • wheat • barley • Carbon isotope discrimination ($\Delta^{13}C$) • water availability

## 6.1. Introduction

Wheat and barley are among the first cultivated crops, and its cultivation has been successively improved across millennia, through a combination of genetic selection and advances in agronomic techniques. Grain yield (i.e. total weight of grains per unit of cultivated area) is currently the trait of greatest interest for agronomist, and it was crucial for the survival and development of early farming. It is defined by three numerical components: the number of spikes per unit grown area, the number of grains per spike, and individual grain weight (GW). From the current knowledge, however, it is not possible to find direct evidences of the values attained in the past for the first two components, at least until the beginning of writing. Even in this case, existing documentary records are often difficult to interpret, as they are not easily comparable with current agronomic studies. Charred plant remains (including grains of cereals and other crops) are routinely recovered from archaeological sites, and dimensions and other morphometric traits of cereal grains are systematically measured (Buxó 1997; Renfrew 1973; Van Zeist and Casparie 1984). Unfortunately, grain proportions change considerably after charring and, consequently, are not directly comparable with those of intact grains (Hopf 1955; Lone *et al.* 1993). Ferrio *et al.* (2004a) developed a method to estimate the original GW of cereal remains from the dimensions of charred grains, which was robust enough to override the effect of carbonisation. Unlike grain dimensions, GW is routinely determined by agronomists, and thus its quantification in charred grains allows to interpret archaeobotanical data under the light of existing agronomic knowledge.

Despite being strongly genetically determined, GW also depends on climatic constraints, such as water availability or temperature stress (Calderini *et al.* 1999b; Rharrabti *et al.* 2003; Voltas *et al.* 1999). Specifically, the main factors defining GW are 1) the amount of assimilates (carbohydrates) per grain during grain filling and 2) the duration of the grain filling period. The amount of assimilates per grain depends on plant conditions during the whole growth cycle, but also on the number of grains. If plants become stressed early in the growth cycle, both the assimilates and the number of grains are reduced and individual grain size may remain unaffected (Henry and Kettlewell 1996). However, when crops are initially grown in favourable conditions, but become stressed during grain filling (which is a common case in Mediterranean environments), the time span for grain growth is reduced and grain size is strongly limited (Gooding *et al.* 2003; Savin and Molina-Cano 2002). Thus, changes in grain size of cultivated cereals in the past may be due to either environmental or genetic factors, or a combination of both.

Ferrio *et al.* (2004a) applied their models to estimate GW over a set of archaeological seed remains from the Segre and Cinca Valley (Catalonia, NE Spain), and they found significantly lower GW during the period studied (*ca*. 2000-200 BCE) than in present times. They attributed such differences in GW mostly to genetic changes attained since the end of the first millennia BCE, based on several palaeoenvironmental evidences indicating that climate during the studied period was more humid than present. However, before discarding the effect of climatic constraints on grain size, it would be helpful to obtain more direct evidences of the water status in which archaeological grains were developed, as it often limits GW (Gooding *et al.* 2003; Savin and Molina-Cano 2002). For $C_3$ species, $\delta^{13}C$ in plant tissues constitutes an integrated record of the ratio of intercellular to atmospheric partial pressure of $CO_2$ ($c_i/c_a$) during the time the tissue was synthesized. In winter cereals, this ratio is mainly determined by stomatal conductance and hence $\delta^{13}C$ in grains represents a good indicator of water status during grain filling. Accordingly, the measurement of $\delta^{13}C$ from charred grains found in archaeological sites has been proposed as a method to evaluate water inputs (due to rainfall and/or irrigation practices) in ancient agriculture (Araus *et al.* 1997; Araus *et al.* 1999).

The specific aim of this work was to determine the relative effect of environmental changes on the evolution of GW in the Segre and Cinca Valley during the last four millennia. For this purpose, we performed $\delta^{13}C$ analyses in a set of charred grains, recovered from the same archaeological sites in which grain measurements were taken. In order to refine the timing of the observed changes in GW from the archaeological context to present-times, we also applied the models described in Ferrio et al. (2004a) to estimate GW to new sets of archaeological material from the same area, covering a wider temporal and spatial range.

## 6.2. Materials and Methods

### 6.2.1. Archaeological sites and plant remains

Charred grains of naked wheat (*Triticum aestivum/durum*) and hulled barley (*Hordeum vulgare*) were recovered from nine archaeological sites in the Segre and Cinca Valleys (Mid Ebro Depression, NE Spain, see Fig. 6.1 and Table 6.1), including the temporal range between the Bronze Age (*ca*. 1950 BCE) and Middle Ages (XI c. CE). These sites now display similar climatic and soil conditions, and are characterized by a semi-arid Mediterranean climate. The samples were collected from various archaeological contexts, such as domestic fires, cooking ovens, storage jars, room floors and levels of rubble from housing structures and pits (for further details about the sites and sampling procedures, see Alonso 1992, 1999, 2005; Alonso et al. 2002; Sarró excavation team 2001). For the sites from the Bronze and First Iron Age, radiocarbon ages were determined at Beta Analytical Inc. (Miami, USA) and at the Universitat de Barcelona (Barcelona, Spain), and calibrated dating was calculated with the software CALIB REV 4.3 (Stuiver *et al.* 1998). For the remaining sites, a combination of stratigraphic and archaeological dating was used. To recover plant remains, soil samples were treated using a standard flotation tank in the field with 5 mm, 2 mm and 0.5 mm sieves, and taxonomical determinations were performed on the basis of the morphological features of the grains.

### 6.2.2. Carbon isotope composition and meteorological data

Because of the carbonate enrichment of archaeological sediments, we soaked each grain separately with HCl 6M for 24h at room temperature to remove carbonate crusts (DeNiro and Hastorf 1985). The samples were then rinsed repeatedly with distilled water and oven-dried at 60ºC for 24 hours before milling to fine powder for carbon isotope analyses. $\delta^{13}$C was determined by elemental analysis and isotope ratio mass spectrometry (EA/IRMS) at Isotope Services, Inc., Los Alamos, NM, USA. To account for changes in $\delta^{13}$C of atmospheric $CO_2$ ($\delta^{13}C_{air}$) during the Holocene, we calculated carbon isotope discrimination in grains ($\Delta^{13}C_g$) from $\delta^{13}C_{air}$ and grain $\delta^{13}$C ($\delta^{13}C_g$), as described by Farquhar *et al.* (1982):

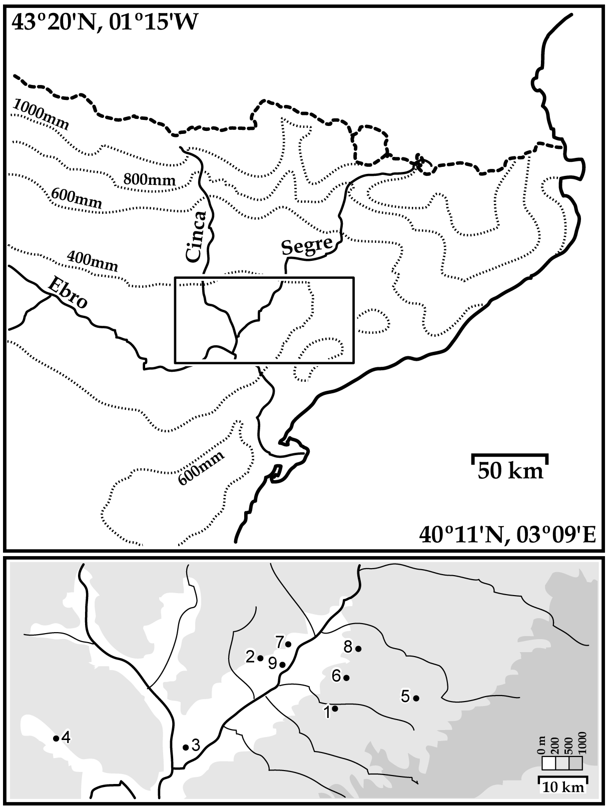

**Fig. 6.1** Geographical location of the archaeological sites included in our study, indicating, in the upper section of the map, the isohyets of mean annual precipitation, and in the lower section, altitude a.s.l.

**Eq. 6.1**

$$\Delta^{13}C_g = \frac{\delta^{13}C_{air} - \delta^{13}C_g}{(1 + \frac{\delta^{13}C_g}{1000})}$$

$\delta^{13}C_{air}$ was inferred by interpolating a range of data from the literature, covering the temporal sample range (Eyer *et al.* 2005; Francey *et al.* 1999; Indermühle *et al.* 1999; Leuenberger *et al.* 1992).

### 6.2.3. Estimation of grain weight

Grain dimensions of wheat and barley grains were determined in all the studied sites (up to one hundred grains per species and stratigraphic unit), but excluding samples too distorted and/or broken to be measured. We measured length (L), breadth (B) and thickness (T) under a stereoscopic microscope with an LCD camera coupled to a PC, assisted by image analysis software (Leika Qwin Lite 2.1, 1997).

GW of archaeological seeds was estimated from the products Length x Breadth (L×B) and Length x Thickness (L×T), taking the mean value of both estimates, as described in Ferrio *et al.* (2004a):

For wheat:

**Eq. 6.2**

$$GW = -15.4 + 2.47 \cdot (L \times B)$$

**Eq. 6.3**

$$GW = -15.1 + 2.98 \cdot (L \times T)$$

For barley:

**Eq. 6.4**

$$GW = 0.21 \times (L \times B)^{1.60}$$

**Eq. 6.5**

$$GW = 0.78 \times (L \times B)^{1.28}$$

### 6.2.4. Estimation of water inputs during grain filling

Total water inputs (TWI) during grain filling were estimated from the $\Delta^{13}C$ of charred grains, following the models defined in Araus *et al.* (1997; 1999), as modified by Ferrio *et al.* (2005a):

For wheat:

**Eq. 6.6**

$$TWI = 0.175 \times e^{(0.376 \times \Delta^{13}\mathrm{C})}$$

For barley:

**Eq. 6.7**

$$TWI = 0.225 \times e^{(0.364 \times \Delta^{13}\mathrm{C})}$$

### 6.2.5. Statistical analyses

We fitted locally weighted least squares regression curves (LOESS, Cleveland 1979) to the data sets to objectively summarize overall trends in the time series of the studied variables (GW, $\Delta^{13}C$). Linear regressions between $\Delta^{13}C$ and estimated GW were built on a subset of samples, in both variables were simultaneously available, to model the effect of water status on grain size. Differences in such relationship between wheat and barley were determined using an heterogeneity of slopes ANOVA. From such regressions, we calculated residual GW values, to quantify the variability not associated to water availability during grain filling. To get a more robust plot of the evolution of GW residuals, missing values of either $\Delta^{13}C$ or GW were filled using the estimations given by LOESS curves.

**Table 6.1** Dating, cultural period, average grain dimensions and estimated grain weight for the archaeological sites (see Fig. 6.1). N, number of grains measured; L × B × T, grain dimensions (length × breadth × thickness); GW, estimated grain weight.

| Archaeological site | Calendar year (BCE/CE) | Cultural Period | Barley | | | Wheat | | |
|---|---|---|---|---|---|---|---|---|
| | | | **N** | **L × B × T (mm)** | **GW (mg)** | **N** | **L × B × T (mm)** | **GW (mg)** |
| 1. Minferri | 1946 cal. BCE | Early Bronze Age | 24 | 4.5 × 2.9 × 2.1 | 14.1 ± 0.9 | 32 | 4.6 × 3.1 × 2.7 | 20.3 ± 0.9 |
| | 1875 cal. BCE | | 1 | 5.7 × 3.2 × 2.4 | 21.3 | 22 | 4.5 × 2.8 × 2.4 | 17.0 ± 1.4 |
| | 1673 cal. BCE | | 3 | 4.5 × 2.3 × 2.0 | 10.9 ± 0.6 | 47 | 4.4 × 2.9 × 2.6 | 17.5 ± 0.8 |
| 2. El Vilot de Montagut | 1412 cal. BCE | Middle Bronze Age | 2 | 4.9 × 2.6 × 2.1 | 13.7 ± 1.0 | 10 | 4.4 × 3.2 × 2.7 | 20.2 ± 1.6 |
| | 888 cal. BCE | Late Bronze Age | 17 | 5.0 × 2.9 × 2.1 | 15.9 ± 1.4 | 13 | 4.4 × 2.9 × 2.4 | 16.8 ± 1.7 |
| | 773 cal. BCE | | 6 | 5.0 × 2.8 × 2.3 | 16.3 ± 1.9 | 1 | 4.7 × 3.0 × 2.4 | 19.1 |
| | 773 cal. BCE | | 68 | 5.5 × 2.9 × 2.3 | 19.0 ± 0.7 | 14 | 4.5 × 2.9 × 2.5 | 17.9 ± 1.4 |
| 3. Masada del Ratón | 1000 cal. BCE | Late Bronze Age | 1 | 4.2 × 2.4 × 1.8 | 9.4 | 4 | 4.3 × 2.6 × 2.4 | 14.7 ± 2.9 |
| 4. Tozal de los Regallos | 896 cal. BCE | First Iron Age | - | - | - | - | - | - |
| | 750 cal. BCE | | 4 | 4.9 × 2.7 × 2.1 | 14.3 ± 2.2 | 1 | 4.3 × 2.3 × 1.8 | 8.4 |
| 5. Els Vilars | 793 cal. BCE | First Iron Age | - | - | - | 3 | 4.6 × 2.6 × 2.2 | 15.2 ± 3.4 |
| | 781 cal. BCE | | 2 | 5.7 × 2.6 × 2.1 | 17.0 ± 4.3 | - | - | - |
| | 500 BCE | Second Iron Age | 170 | 5.6 × 2.9 × 2.3 | 19.8 ± 0.4 | 87 | 4.5 × 2.8 × 2.4 | 16.8 ± 0.6 |
| 6. Margalef | 200 BCE | Second Iron Age | 100 | 5.7 × 2.9 × 2.2 | 19.0 ± 0.3 | 100 | 5.0 × 3.0 × 2.6 | 22.4 ± 0.5 |
| 7. Roques Sarró | 200 BCE | Iron Age | - | - | - | 6 | 4.5 × 2.6 × 2.2 | 14.1 ± 2.3 |
| 8. Tossal de les Tenalles | 200 BCE | Second Iron Age | 100 | 6.1 × 3.0 × 2.4 | 23.2 ± 0.5 | 100 | 5.1 × 2.9 × 2.5 | 21.9 ± 0.4 |
| 9. Ciutat de Lleida | 50 CE | Roman | 2 | 5.9 × 3.0 × 2.3 | 20.7 ± 1.9 | 5 | 4.8 × 3.1 × 2.3 | 19.9 ± 2.0 |
| | 300 CE | | - | - | - | 8 | 4.4 × 3.0 × 2.5 | 17.5 ± 2.3 |
| | 1025 CE | Middle Age | - | - | - | 2 | 5.7 × 3.3 × 2.5 | 28.7 ± 1.9 |
| | 1040 CE | | 6 | 5.7 × 2.7 × 2.1 | 17.6 ± 1.5 | 3 | 4.8 × 3.0 × 2.8 | 22.6 ± 0.5 |
| **Total** | | | **506** | **5.6 × 2.9 × 2.3** | **19.6 ± 0.2** | **458** | **4.7 × 2.9 × 2.5** | **19.6 ± 0.3** |

## 6.3. Results and Discussion

### *6.3.1 Grain weight and carbon isotope values in archaeological material*

Grain dimensions of charred seeds showed a considerable range of variation across archaeological sites (see Table 6.1), ranging from 9.4 to 23.2 mg in the case of barley, and from 8.4 to 28.7 mg for wheat. When using regression models to estimate a given variable, reliable estimations are only obtained for samples lying within within the range of variation used for calibration. In our case, over 95% of the grains were well inside the ranges of L×B and L×T used by Ferrio *et al.* (2004a) to calibrate GW models (Eqs. 6.2-6.3), indicating that such models are appropriate for the present data. Estimated GW in archaeological grains (19.6 mg in average for both species) was considerably smaller than the values found in present rainfed cereals cultivated in the area (30 mg ±1.5 for barley and 35 mg ± 2.0 for wheat, according to Ferrio *et al.* (2004a).

Average $\delta^{13}C$ in grains for the sites studied also varied considerably, ranging from -23.4 to -21.1 ‰ in barley, and from -23.0 and -20.8 ‰ in wheat (Table 6.2). Estimated $\delta^{13}C$ in atmospheric $CO_2$ suffered relatively small changes during the period considered, and thus calculated $\Delta^{13}C$ showed nearly the same range of variability found for $\delta^{13}C$

(from 14.8 to 17.4 ‰, and from 14.2 to 17.0, for barley and wheat, respectively). On average, $\Delta^{13}C$ in barley grains was higher than in wheat (16.1 ‰ ±0.2 and 15.7 ‰ ±0.2, respectively). The same general trend has been reported in previous studies on archaeological material collected around the Mediterranean (Araus *et al.* 1997; Araus *et al.* 1999; Ferrio *et al.* 2005a). Such differences in isotope signature can be attributed to the shorter growing cycle of barley, which reaches maturity about two weeks earlier than wheat. As Mediterranean climates are characterised by a steep decrease in water availability (and rising temperatures) by the end of the cereals growing cycle (late spring), plants with shorter cycle would suffer less water stress during grain filling, which is translated into lower $\Delta^{13}C$ in grains. In both cases, the observed range of $\Delta^{13}C$ felt within the calibration range of the models used to predict TWI (Eq. 6.6-6.7, see Araus *et al.* 1997; 1999; Ferrio *et al.* 2005a), implying that these models can give reliable estimations of TWI for our samples. Estimated values of TWI were highly variable, but they are still within the range of values reported for rainfed cereals cultivated around the archaeological sites: between *ca.* 30-150 mm for barley (Araus *et al.* 1997; Voltas *et al.* 1997) and between *ca.* 50-110 mm for wheat (Araus *et al.* 2003; Ferrio *et al.* 2005b). Moreover, average TWI in archaeological grains did not differ significantly from the estimations derived from present $\Delta^{13}C$ values in grains (82 mm ±14 for barley and 62 mm ±20 for wheat, after (Ferrio *et al.* 2005a). It should be noted that, despite showing differences in $\Delta^{13}C$, estimations of TWI for archaeological wheat and barley did not differ significantly. This means that, for a given $\Delta^{13}C$, TWI in wheat would be greater than in barley. Again, this would agree with the shorter cycle of barley: even receiving the same amounts of water during grain filling (i.e. TWI), wheat plants would become more stressed during this period because of a greater vapour pressure deficit, driven by higher temperatures.

**Table 6.2** Dating, estimated $\delta^{13}C$ in atmospheric $CO_2$ ($\delta^{13}C_{air}$), average carbon isotope composition ($\delta^{13}C$), discrimination ($\Delta^{13}C$), and estimated water inputs during grain filling (TWI) in charred grains recovered from each archaeological sites.

| Archaeological site | Calendar year (BCE/CE) | $\delta^{13}C_{air}$ (‰) | Barley | | | | Wheat | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | $\delta^{13}C$ (‰) | $\Delta^{13}C$ (‰) | TWI (mm) | N | $\delta^{13}C$ (‰) | $\Delta^{13}C$ (‰) | TWI (mm) |
| 1. Minferri | 1946 cal. BCE | -6.36 | 2 | -22.4 ± 0.0 | 16.4 ± 0.0 | 82 ± 0 | 2 | -22.4 ± 0.0 | 16.4 ± 0.0 | 88 ± 1 |
| | 1673 cal. BCE | -6.42 | 2 | -22.2 ± 0.6 | 16.1 ± 0.6 | 76 ± 18 | 2 | -20.8 ± 0.0 | 14.7 ± 0.0 | 47 ± 0 |
| 2. El Vilot de Montagut | 1412 cal. BCE | -6.47 | 2 | -20.9 ± 0.2 | 14.8 ± 0.2 | 23 ± 2 | 2 | -21.7 ± 0.0 | 15.6 ± 0.0 | 66 ± 0 |
| | 888 cal. BCE | -6.54 | 2 | -22.9 ± 0.3 | 16.7 ± 0.4 | 86 ± 20 | 2 | -22.1 ± 0.0 | 15.9 ± 0.0 | 73 ± 0 |
| | 773 cal. BCE | -6.54 | 2 | -22.6 ± 0.1 | 16.4 ± 0.1 | 69 ± 5 | 2 | -22.2 ± 0.1 | 16.0 ± 0.1 | 77 ± 2 |
| 3. Masada R. | 1000 cal. BCE | -6.53 | 2 | -21.6 ± 0.5 | 15.4 ± 0.5 | 58 ± 10 | 1 | -22.1 | 16.0 | 75 |
| 4. Tozal R. | 896 cal. BCE | -6.54 | 2 | -21.8 ± 0.1 | 15.6 ± 0.1 | 40 ± 3 | - | - | - | - |
| 5. Els Vilars | 793 cal. BCE | -6.54 | 2 | -22.3 ± 0.9 | 16.2 ± 1.0 | 71 ± 40 | 2 | -21.2 ± 0.1 | 15.0 ± 0.1 | 52 ± 1 |
| | 781 cal. BCE | -6.54 | 2 | -21.8 ± 0.0 | 15.6 ± 0.0 | 40 ± 1 | 1 | -20.9 | 14.6 | 46 |
| | 500 BCE | -6.59 | 2 | -21.1 ± 0.0 | 15.0 ± 0.0 | 27 ± 0 | 2 | -20.4 ± 0.0 | 14.2 ± 0.0 | 40 ± 0 |
| 6. Margalef | 200 BCE | -6.45 | 2 | -23.4 ± 0.0 | 17.4 ± 0.0 | 120 ± 1 | 2 | -22.0 ± 0.0 | 15.9 ± 0.0 | 73 ± 0 |
| 7. R. Sarró | 200 BCE | -6.45 | - | - | - | - | 2 | -21.6 ± 0.6 | 15.5 ± 0.6 | 65 ± 14 |
| 8. T. Tenalles | 200 BCE | -6.45 | 2 | -23.0 ± 0.2 | 17.0 ± 0.2 | 100 ± 15 | 2 | -21.9 ± 0.0 | 15.8 ± 0.0 | 71 ± 0 |
| 9. Lleida | 300 CE | -6.36 | 2 | -22.2 ± 0.1 | 16.2 ± 0.1 | 76 ± 3 | 2 | -23.0 ± 0.2 | 17.0 ± 0.2 | 110 ± 9 |
| 9. Lleida | 1025 CE | -6.42 | 1 | -22.6 | 16.5 | 86 | 1 | -23.0 | 17.0 | 108 |
| **Total** | | | **27** | **-22.2 ± 0.2** | **16.1 ± 0.2** | **68 ± 6** | **25** | **-21.8 ± 0.2** | **15.7 ± 0.2** | **70 ± 4** |

### 6.3.2 Evolution of carbon isotopes and grain weight during the last 4000 years

In general terms, wheat and barley showed similar trends throughout the studied period for $\Delta^{13}C$ (Fig. 6.2a). $\Delta^{13}C$ was similar to current values during the second millennium BCE, showing a strong depletion from *ca.* 800 BCE to 500 BCE. After this, a significant recovery is apparent from *ca.* 300 BCE, probably spanning to the $XI^{th}$ century BCE. From the $XI^{th}$ century to present times, wheat showed certain decrease in $\Delta^{13}C$, whereas barley maintained similar values from *ca.* 300 BCE to present.

In a similar way, wheat and barley followed nearly the same pattern for the evolution of GW during the last four millennia (Fig. 6.2b). However, such pattern is contrastingly different from that found for $\Delta^{13}C$. Present GW (30-35 mg) was far greater than the estimations obtained for archaeological material, which barely exceeded the 20 mg threshold from *ca* 2000 BCE to 500 CE). We found a relatively constant increment in grain weight during the first millennium BCE, followed by a steeper increase from ca. 500 CE to present times.

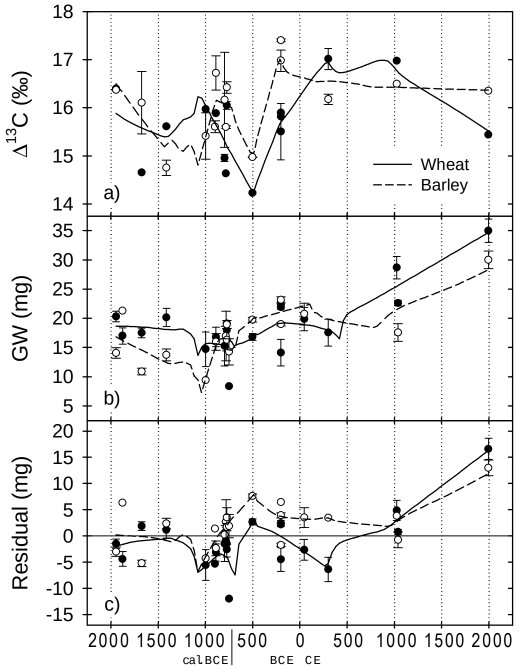

**Fig. 6.2** Evolution of a) carbon isotope discrimination ($\Delta^{13}C$) and b) estimated grain weight (GW) in wheat (filled circles) and barley (empty circles) during the last four millennia. As a reference, present values of $\Delta^{13}C$ {Ferrio, Araus, et al. 2005 6491 /id} and grain weight {Ferrio, Alonso, et al. 2004 5677 /id} in the area are plotted. c) residuals resulting from the linear regression between GW and $\Delta^{13}C$ (Eq. 6.8). Trend lines depict locally weighted least squares regression curves (LOESS, *span*=0.4) fitted to the data. Vertical error bars indicate the standard error of the mean.

### 6.3.3 To what extent water stress conditioned grain weight in ancient crops?

GW is a character with a strong genetic basis, and thus its evolution with time might respond to either genetic or environmental changes. Although, in some cases, DNA can be recovered from archaeological charred grains (see e.g. Brown 1999; Schlumbaum *et al.* 1998), systematic studies of the genetic pool of such material are still not feasible. Alternatively, we can quantify the potential role of environmental conditions (at least those related with water availability) by comparing $\Delta^{13}C$ and GW evolution. Firstly, we took a subset of 26 archaeological samples (14 for wheat 12 for barley) in which pairwise comparisons between both variables were possible. We found a weak, but significant relationship between $\Delta^{13}C$ and GW for wheat and barley. The heterogeneity of slopes ANOVA revealed that the slope of the resulting regression lines did not differ significantly for the two species, but they had distinct intercepts. Thus, we assumed equal slopes for the two species, and fitted the following model, including barley and wheat data:

**Eq. 6.8**

$$GW = -40.7 + 3.53 \times \Delta^{13}\mathrm{C} + 4.6 \times (sp = wheat)$$
$$r^2 = 0.40, P < 0.01$$

where *sp=wheat* is an indicator variable which takes the value 1 if true and 0 if false, in order to modify the intercept of the regression, according to the species. We subsequently applied this model to all the studied sites, and the residuals between GW (calculated from grain dimensions) and GW predicted by $\Delta^{13}C$ were plotted (Fig. 6.2c). The departure from the zero line of the residuals would be an indicator of GW changes that are not attributable to changes in water availability. The most clear trend in the evolution of residual GW is a great increase from archaeological to modern material. Such increment might have been started during Middle Age, but still during this period GW values were significantly lower than in present-times. Whereas the residual for archaeological grains oscillated around zero (± 5 mg), GW in modern material was much higher than the expected values derived from Eq. 6.8 (+19.0 mg and +13.1 mg, for wheat and barley, respectively). Indeed, the residual plot have enhanced the observed increment in GW for modern crops, which contrasted with the relatively stable $\Delta^{13}C$ values. At this point, we should consider the possibility that grain weight in archaeological sites was limited by environmental constraints others than water availability. For example, both pre- or post-anthesis temperature stress may decrease strongly final grain weight (Calderini *et al.* 1999a; Calderini *et al.* 1999b). However, under semi-arid climates, higher temperatures should be also translated into lower $\Delta^{13}C$ in plants, due to an increase in vapour pressure deficit (Condon *et al.* 1992; Ferrio and Voltas 2005; Zhang and Nobel 1996). This was not our case, as we found an average $\Delta^{13}C$ in archaeological sites (see Table 6.2) similar to that found in present crops (16.4‰ ±0.5 for barley and 15.4‰ ±0.9 for wheat). Moreover, several palaeoenvironmental evidences indicate a global rise in temperature since mid XIXth century (Barriendos and Martín-Vide 1998; Creus *et al.* 1996; Folland *et al.* 2001; Riera *et al.* 2004), further discarding the possibility of higher temperature stress in the past. Alternatively, current agronomic practices, like the extensive application of nitrogen fertilisers, might be also responsive of changes in grain size. However, although poor nitrogen status may be as important as water scarcity in limiting crop performance in Mediterranean climates (Passioura 2002), the effect of nitrogen availability on grain weight is less clear. Nitrogen starvation tends to decrease the number of grains per plant, but has little effect on individual grain weight (Oscarsom *et al.* 1999) and in some cases can lead to greater grain weights, due to compensation mechanisms (Corbeels *et al.* 1999; Ferrio *et al.* 2004b).

The apparent lack of environmental constraints (at least in terms of water availability) underlying the lower grain size of archaeological grains, compared with modern material, suggest that it was determined by genetic factors. Such genetic gain in grain weight cannot be attributed, however, to modern plant breeding programs, as traditional landraces and modern cultivars show similar values for this trait (Abeledo *et al.* 2002; Cascón 1934). Indeed, grain size might have been among the first selection criteria used by farmers. Greater grains show higher flour yield and lower bran content, thus affecting their nutritive value (Henry and Kettlewell 1996; Wiersma *et al.* 2001), and also have better malting quality in barley (Molina-Cano *et al.* 1997) and baking properties in wheat (Evers *et al.* 1995). Moreover, grain weight is also positively associated with germination power (Mian and Nafzinger 1994) and early vigour (López-Castañeda *et al.* 1996; Richards and Lukacs 2002), ensuring a better development, specially for the harsher environmental conditions (Grieve and François 1992; Mian and Nafzinger 1994). Thus, it is quite probable that selecting the biggest seeds for sowing was a well-known practice among ancient farmers (Cascón 1934; Zeven 2000).

### 6.3.4 Climatic and genetic effects on the water status of cereal crops

To further assess the environmental background in which archaeological crops were developed, we compared the evolution of TWI in cereals with estimated annual precipitation (Fig. 6.3), derived from $\Delta^{13}C$ analyses of charcoal fragments of Aleppo pine (*Pinus halepensis* Mill.), recovered in the same sites (Ferrio, Alonso, López, Araus and Voltas, submitted manuscript). The climatic reconstruction derived from forest trees allows us to determine the role of general climatic

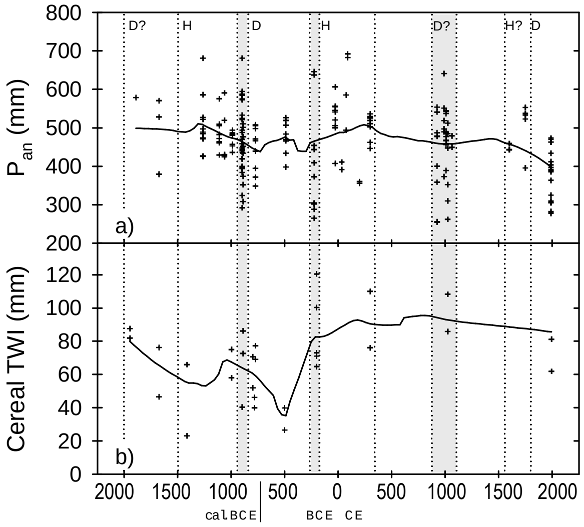

**Fig. 6.3** Comparison between the evolution of estimated mean annual precipitation ($P_{an}$, Ferrio *et al.*, submitted) and total water inputs during grain filling (TWI) in cereals (wheat and barley combined) in the studied area. $P_{an}$ was estimated from carbon isotope discrimination ($\Delta^{13}C$) of *Pinus halepensis* charcoal in the same sites used in the present work. Trend lines depict locally weighted least squares regression curves (LOESS, *span*=0.3 for Pan, *span*=0.4 for TWI) fitted to the data. Relatively dry (D) and humid (H) phases (according to Ferrio *et al.*) are indicated, as well as periods of strong climatic variability (shadow areas).

trends on the observed changes in cereal TWI. Due to the small amount of sample available, the period from *ca.* 2000-1000 BCE was poorly characterized for the cereals, and thus the results are not fully comparable. Nevertheless, in both cases the driest period, among archaeological samples, was placed between *ca.* 800 BCE and 500 BCE, and it was followed by a recovery from ca. 300 BCE to 300 CE. The subsequent period of strong climatic variability around 1000 CE could not be contrasted in the cereals, as only two samples of cereal were analysed for this period. Intriguingly, whereas estimates of annual precipitation in the past (from wood $\Delta^{13}C$) were consistently higher than in present-times, this was not the case for cereal TWI. The most probable explanation for such divergence could rely on the recent genetic change of cereals resulting from plant breeding programs. Modern cultivars have been usually selected to attain better performance under optimal conditions (i.e. to maximise yield potential), being less conservative in terms of water expense (Araus *et al.* 2002). This is translated into lower stomatal limitation of photosynthesis, and thus higher $\Delta^{13}C$ than traditional landraces when growing under the same conditions (Fischer *et al.* 1998; Zhao *et al.* 2001), with differences of up to 1‰. Moreover, the varieties released since 1960 are characterised by an overall reduction in plant height and growing cycle, and hence the grains are developed under cooler and more humid conditions, contributing to the increase $\Delta^{13}C$ (Abeledo *et al.* 2002; Araus *et al.* 2002). Accordingly, it would be helpful to compile more reference data from traditional landraces, in order to make proper comparisons between archaeological and present-times.

## 6.4. Conclusions

Our results confirm that, in the area of study, grain weight was significantly smaller in the past than in present-times. We found an overall trend of increasing grain weight since *ca.* 1000 BCE, becoming steeper from ca. 1000 CE to present times. According to $\Delta^{13}C$ data, the most recent increase in grain weight was not associated to increased water availability. Consequently, we concluded that the greater grain weight in present-day cereals responded to a genetic improvement of the crops, which might have started sometime from Roman Period. The general agreement between estimations of annual precipitation, derived from fossil charcoal, and estimated water inputs in cereals, indicates that the water status of such crops was mostly determined by climatic constraints. Nevertheless, modern material used for reference caused an overestimation of present water status, probably due to the greater stomatal conductance associated to modern varieties.

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# General Discussion

*"The most merciful thing in the world, I think, is the inability of the human mind to correlate all its contents."*

H.P. Lovecraft. *The Call of Cthulu*

## I. Different roads to Rome: evolutionary keys for the divergences between Aleppo pine and Holm oak in response to drought

The temporal uncoupling of temperature and precipitation, with the driest period coinciding with the highest temperatures, as well as the existence of nutrient-deficient soils have been major evolutionary forces in shaping Mediterranean plant communities (Herrera 1992; Mooney and Dunn 1970). Examples of common adaptations include extensive root systems, evergreen foliage to compensate for nutrient scarcity and to permit year-round production, water-saving mechanisms such as leaf sclerophylly or a very sensitive stomatal regulation (Rundel 1988; Zavala *et al.* 2000). The results presented in Chapter 1 confirm that, although both Aleppo pine (*P. halepensis* Mill.) and Holm oak (*Quercus ilex* L.) are well adapted to Mediterranean climates, the strategies adopted by each species differ considerably. *P. halepensis* is a typical drought-avoiding species, which concentrates most of their response to drought on water saving (mostly through stomatal closure), but acts as an opportunistic species when conditions are favourable. On the other hand, *Q. ilex* is a slow growing, deep-rooted species that, although showing a considerable stomatal regulation (at least when compared to other species of the same genus), shows a very effective water uptake, together with drought-tolerance mechanisms, such as osmotic adjustment. This explains the strong response to water availability of carbon isotope discrimination ($\Delta^{13}C$) and radial growth in *P. halepensis*, contrasting with the weaker sensitivity of *Q. ilex*. The apparent lack of sensitivity in radial growth of *Q. ilex*, however, contrasts with the results of studies of the temporal variability of tree-rings (Corcuera *et al.* 2004). Over the great spatial gradient here studied, several compensation mechanisms might have minimised long-term variability in radial growth: changes in phenology, different patterns of assimilate distribution between leaves and xylem (Villar-Salvador *et al.* 1997). Such compensation mechanisms might result from either acclimation processes (Villar-Salvador *et al.* 1998; Villar-Salvador *et al.* 1999) or genetic differences between provenance regions (Gratani *et al.* 2003; Tognetti *et al.* 1997).

$\Delta^{13}C$ values in *Q. ilex*, moreover to having lower sensitivity to water availability, were consistently higher than those found in *P. halepensis* (see Fig. 1.3). Previous studies on co-occurring conifers and oaks have also shown that the former tend to show lower $\Delta^{13}C$ than the latter (Picon *et al.* 1996; Valentini *et al.* 1992). Differences in $\Delta^{13}C$ between *Q. ilex* and *P. halepensis*, for the common range in the ratio between precipitation and evapotranspirative demand (P/E = 0.45-1.08), ranged from 0.7‰ to 1.5‰, being higher in drier environments. Thus, for the same levels of aridity, *P. halepensis* displays stronger stomatal limitation of photosynthesis, and consequently higher water use efficiency ($WUE_i$) and lower $\Delta^{13}C$, and this difference increases in response to water stress. At this point, we should bear in mind that the proportion of lignin and cellulose, as well as the metabolic routes for the synthesis of lignin, are different in oaks and conifers, and this might cause differences in $\Delta^{13}C$ between species, not related with $WUE_i$ (Barbour *et al.* 2001; Borella *et al.* 1998), see also Chapter 2). Nevertheless, when comparing $\Delta^{13}C$ in holocellulose for the two species (see Chapter 2, and Vega 2003 for data on *Q. ilex*) we found the same pattern observed for wood, and the absolute differences were even higher (from 0.8‰ to 1.8‰, over the same range of P/E). All these results confirm that *Q. ilex*, despite being a species with a strong capacity of stomatal regulation (Damesin *et al.* 1998; Infante *et al.* 1999), should be considered as a partially drought tolerant species, which also relies on other mechanisms to cope with drought. Moreover, to be able to extract water from far deeper soil layers, the threshold of predawn water potential for stomatal closure for *Q. ilex* is much lower than for *P. halepensis* (Borghetti *et al.* 1998; Damesin *et al.* 1998). Consequently, *Q. ilex* can show, over the growth season, lower stomatal limitation to photosynthesis than a drought-avoiding conifer such as *P. halepensis*. Our current work provides additional evidences on the physiological differences between the two species, using combined analyses of carbon and oxygen stable isotopes. According to the conceptual model proposed by Scheidegger *et al.* (2000), we hypothesized in Chapter 2 that, in a species with a strong variability in stomatal conductance (e.g. *P. halepensis*), the oxygen isotope composition in wood holocellulose ($\delta^{18}O_C$) would be mostly associated with the water status of trees, rather than with the temperature signal derived from the isotopic composition of rainfall ($\delta^{18}O_R$). Conversely, a species showing alternative strategies to cope with drought, as is the case for *Q. ilex*, would display $\delta^{18}O_C$ more closely related to $\delta^{18}O_R$. We have found that, unlike *P. halepensis*, $\delta^{18}O_C$ in *Q. ilex* (over 15 of the sites described in Chapter 1) can be correlated with temperature (r= 0.58, $P<0.05$) but not with vapour pressure deficit (Ferrio and Voltas, unpublished results). Moreover, no relationship at all was found between $\delta^{13}C$ and $\delta^{18}O$ values in holocellulose for *Q. ilex* (r=-0.01), contrasting with the weak, positive trend described for *P. halepensis* (r=0.36, $P<0.1$). Although $\delta^{18}O$ results for *Q. ilex* are still preliminary, they suggest that $\delta^{18}O_C$ variability across sites for this species cannot be attributed to evaporative enrichment of leaf water, as happened in *P. halepensis*, being mostly determined by source water. Again, this is a result of the lower stomatal control of *Q. ilex*, in relation to *P. halepensis*.

Some of the abovementioned divergences might derive from the different evolutionary history of *P. halepensis* and *Q. ilex*. According to Herrera (1992), life-history traits of Mediterranean species result from a combination of responses to evolutionary processes acting on pre-Mediterranean lineages. Thus, the opportunities to adopt a given adaptive strategy to Mediterranean-type climates are restricted by the particular morphological and physiological traits of each evolutionary line. In extreme cases, certain groups, too adapted to previous environmental conditions, disappeared from the Mediterranean area. Other groups succeeded to develop new drought-adapted forms, but through mechanisms constrained by their primitive traits. In the case of *P. halepensis*, it shares with other conifers the primitive xeromorphic characters of the group (e.g. low surface/volume ratio of leaves), which appeared during the increase in aridity occurring *ca.* 300 My BP, at Permian (Thomas and Spicer 1987). Moreover, the thermophil nature and indefinite growth of *P. halepensis* might also be a primitive character from the sect. *Pinea* (including *P. pinea*, *P. tropicalis* and *P. brutia*), which diverged from the sect. *Sylvestres* in Lower Cretacean, about 120 My BP (Klaus 1987). The former was located in the euro-caribbean region, growing under warm, sub-humid to semi-arid climates, whereas the latter was restricted to higher, colder latitudes. The first fossil appearance of *P. halepensis* occurs during the Eocene (50 My BP), a period characterised by an increase in aridity around Southern Europe (Klaus 1987; Palamarev 1987). Thus, the evolutionary history of *P. halepensis* is marked by successive environmental pressures towards an adaptation to dry, warm climates. On the other hand, the potential ancestors of *Q. ilex* appeared at the end of the Upper Cretacean (70 My BP) in the form of evergreen, laurophyllous species within the paleotropical geoflora, and associated with warm, humid environments (Mai 1987; Manos and Stanford 2001). The first appearance of distinctive traits of *Q. ilex* coincides with the increase in continentality that occurred during the Oligocene (35 My BP), which might explain the presence of winter dormancy in this species (Mai 1987; Palamarev 1987). Indeed, the first fossil remains of *Q. ilex* appear associated with a new type of vegetation, the “mixed mesophytic forest”, together with deciduous species from arctoboreal origin (Mai 1987). During this period, *Q. ilex* was mostly found in continental environments, forming closed forest, whereas *P. halepensis* occupied mostly open spaces in coastal areas (Barrón 2004), thus resembling current habitat preferences (see Fig. VIII). Nowadays, *P. halepensis* is clearly better adapted than *Q. ilex*

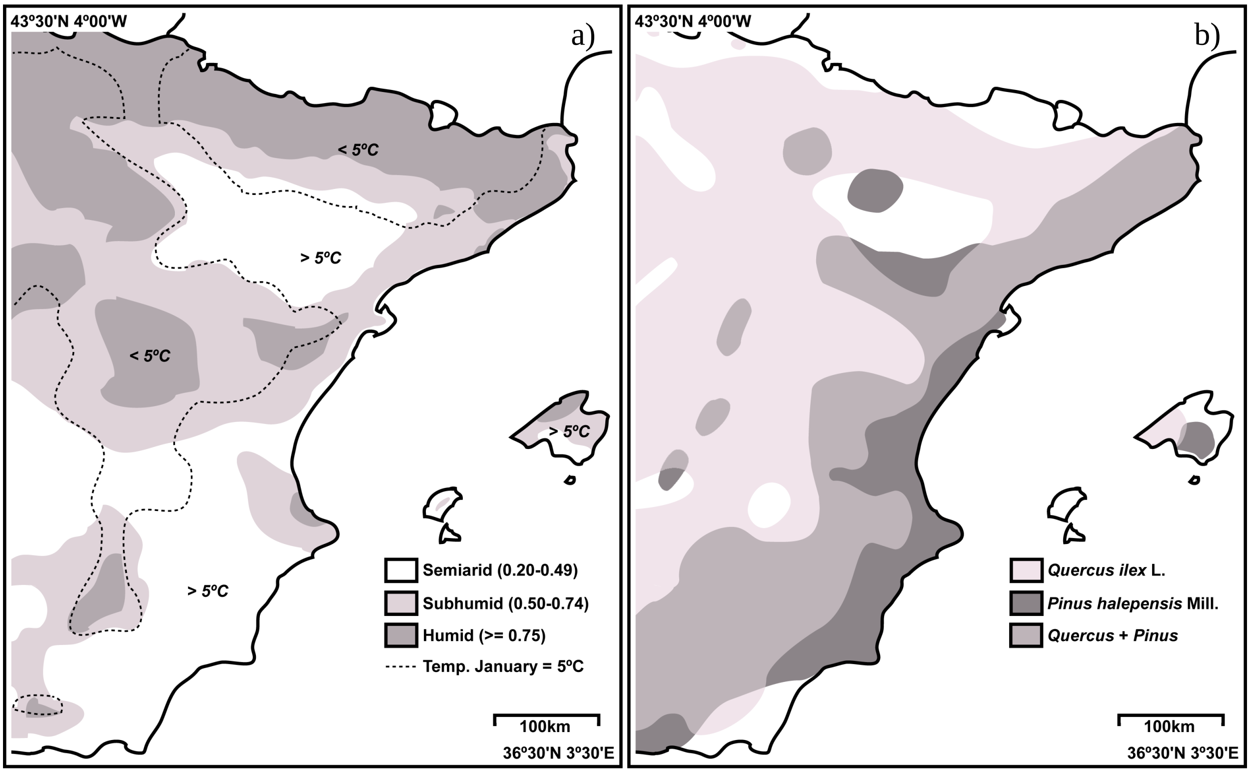


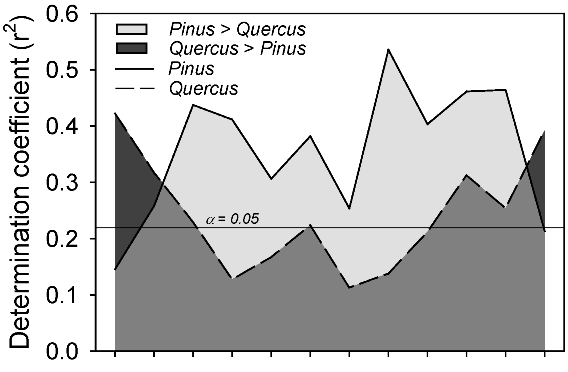
**Fig. VIII** Relationship between climate and potential distribution of *Quercus ilex* L. and *Pinus halepensis* Mill. in Eastern Spain. a) Main bioclimatic regions, according to the UNESCO humidity index (total precipitation/Pemman evapotranspirative demand), and isotherm of 5ºC from January average temperature. Adapted from Spanish Ministry of the Environment (2000) and IGN (1992). b) Climacic distribution of the two species. Adapted from IGN (1995).

to the typical (semi-arid) Mediterranean climate of the Iberian Levant and Southern Mallorca, but is rapidly replaced by the latter in wetter environments favouring the formation of dense, dark forests (e.g. Northern Catalonia, Western Mallorca), as well as in the continental Mediterranean climates of the Central Plateau. In such context, some potential adaptations of *Q. ilex* to drought might originally come from the need to reduce the effect of fire events and hervibores, rather than from a drier climate (e.g. hard, sclerophyllous leaves, deep-roots to allow sprouting). Summarising, the evolutionary history of the two species indicates that the great response to drought of *P. halepensis* is derived from successive selection processes, associated with dry episodes, whereas for *Q. ilex* the adaptation to Mediterranean-type climates is relatively recent, and probably linked to other environmental and/or biotic constraints.

## II. Sensitivity to seasonal climate of stable isotopes in tree-rings: implications for (palaeo)environmental studies

Throughout the year, the periods in which precipitation events have a significant effect on the isotopic signal of wood differ substantially between *Q. ilex* and *P. halepensis*. In the case of *P. halepensis*, the good sensitivity of $\Delta^{13}C$ to changes in precipitation nearly during the whole year is compatible with the indeterminate growth of this species (Liphschitz and Lev-Yadun 1986; Nicault *et al.* 2001). Indeed, cambium activity in *P. halepensis* can span over the whole year under favourable conditions, although can be slowed during the driest weeks of summer, as well as in cold winters. Indeed, we found the lowest correlations between monthly precipitation and $\Delta^{13}C$ in January and July, coinciding with the coldest and the driest month of the year, respectively (see Fig IXa). Thus, for *P. halepensis*, the periods of greater sensitivity to climate variables coincide with the expected periods of active growth. This seems to be the case also for other conifers. Indeed, the seasonal pattern of isotopic composition in wood of *Pinus* species has shown to be mostly determined by the environmental conditions in which each wood layer was formed (Barbour *et al.* 2002; Jäggi *et al.* 2003; Leavitt and Long 1991; Schulze *et al.* 2004). For *Q. ilex*, however, the seasonal variation in the sensitivity of bulk wood material to precipitation contrasts with the phenology of this species. Unlike *P. halepensis*, *Q. ilex* has a true dormancy period during winter, as well as a period of reduced growth in summer (Liphschitz and Lev-Yadun 1986). Interestingly, the time in which precipitation has the greater effect on the $\Delta^{13}C$ of wood correspond in part with the period of winter dormancy. Winter rains, although occurring during cambium dormancy, can feed deep-soil water reservoirs, which in turn increases effective water availability during the growing period for a deep-rooting species, such as *Q. ilex*. On the other hand, we cannot rule out the possibility that early wood formation during spring could rely on the remobilisation of photosynthates synthesised during autumn and early winter. Remobilisation of stored carbohydrates is a necessary feature in deciduous trees, as many of them start wood growth before leaf emergence. Recent studies on radial sub-sections of tree-rings have shown that this process may alter significantly the isotopic signal of early wood, precluding its use as an environmental indicator (Helle and Schleser 2004). However, there is a lack of such kind of studies on evergreen, sclerophyllous species such as *Q. ilex*. Currently, we are preparing a study to compare the isotopic seasonal pattern in different co-occuring species from the genus *Quercus*, covering the existing range of leaf habits, from the evergreen *Q. ilex* to the deciduous *Q. petraea*, going through the marcescents *Q. faginea* and *Q. humilis*. The objective of this work will be to find out whether such "isotope anomaly" of early wood is limited to deciduous trees, or it is also present in evergreen species from the genus.

Despite the aforesaid limitations, the differences in the response to seasonal rainfall between species should not be considered only as a handicap for the use of stable isotopes in tree-rings as (palaeo)environmental indicators. Quite the opposite, it is an interesting feature that, provided a good physiological knowledge of the studied material, can provide additional environmental information by combining data from different species. In our case, *P. halepensis* appears, at first glance, as the most suitable species to attain climatic inferences from wood $\Delta^{13}C$, as it is sensitive to precipitation events occurring throughout the year. However, if we compare the response to seasonal rainfall in stands of *P. halepensis* and *Q. ilex* within the same geographic area, the divergences between the two species can be used as an indicator of changes in seasonality. For example, as *P. halepensis* is sensitive to winter colds, and has a poor ability to exploit winter-fed water reserves in the soil, an increase in winter precipitations, accompanied with a descent in temperature, would not cause an increase in $\Delta^{13}C$. Indeed, it might cause a decrease in bulk $\Delta^{13}C$, as a greater portion of wood is synthesised during spring/summer. In contrast, *Q. ilex* would be clearly favoured by this situation: the augment in deep-soil water would increase effective water availability for *Q. ilex*, leading to an increase in the $\Delta^{13}C$ of wood. Thus, under such situation, we would find relatively high $\Delta^{13}C$ in *Q. ilex*, but not in *P. halepensis* (see Fig. IXb). Similarly, an increase in summer storms would cause a positive response in the opportunistic *P. halepensis*, while having little effect on *Q. ilex*. Such kind of conceptual approach might be useful to clarify the real nature of certain climatic anomalies found in the palaeoenvironmental record: the next step would be the transformation of this conceptual model into a quantitative one (calibrated with data from mixed forests), in order to maximise the information obtained from the two species.



a)

| **Stable isotopes** | $P_{spring}$ | $P_{summer}$ | $P_{autumn}$ | $P_{winter}$ |
|---|---|---|---|---|
| $\uparrow \Delta^{13}C_{Pinus} \uparrow \Delta^{13}C_{Quercus}$ | ↑ | ↑ | ↑ | ↑ |
| $\approx \Delta^{13}C_{Pinus} \approx \Delta^{13}C_{Quercus}$ | ≈ | ≈ | ≈ | ≈ |
| $\downarrow \Delta^{13}C_{Pinus} \downarrow \Delta^{13}C_{Quercus}$ | ↓ | ↓ | ↓ | ↓ |
| $\uparrow \Delta^{13}C_{Pinus} \approx \Delta^{13}C_{Quercus}$ | ≈ | ↑ | ≈ | ≈ |
| $\approx \Delta^{13}C_{Pinus} \uparrow \Delta^{13}C_{Quercus}$ | ≈ | ≈ | ≈ | ↑ |
| $\uparrow \Delta^{13}C_{Pinus} \downarrow \Delta^{13}C_{Quercus}$ | ↓ | ↑↑ | ↓ | ↓ |
| $\downarrow \Delta^{13}C_{Pinus} \uparrow \Delta^{13}C_{Quercus}$ | ↓ | ↓ | ↓ | ↑↑ |

b)

**Fig. IX** a) Comparison of the determination coefficients ($r^2$) between carbon isotope discrimination ($\Delta^{13}C$) in wood and monthly precipitation for *Quercus ilex* and *Pinus halepensis* (data from Chapter 1). b) Proposed conceptual model for the interpretation of $\Delta^{13}C$ values in co-occuring *Q. ilex* and *P. halepensis*, according to their differential response to seasonal precipitation. $\Delta^{13}C_{Pinus}$, $\Delta^{13}C_{Quercus}$, $\Delta^{13}C$ in wood of *P. halepensis* and *Q. ilex*, respectively; $P_{spring}$, $P_{summer}$, $P_{autumn}$, $P_{winter}$; total seasonal precipitation; ↑, increasing variable; ≈, steady variable; ↓, decreasing variable; $\alpha$ = *0.05*, threshold line for significance at the 5%.

### *Reinterpreting the "Cold Iron Age Epoch" in the Ebro Basin on the light of $\Delta^{13}$C data*

We can take advantage of this conceptual model to further discuss the environmental conditions in the Segre and Cinca Valleys during the so called *Cold Iron Age Epoch* (*ca.* 900-300 BCE). (Gribbin and Lamb 1978) defined this period, estimating a decrease in temperature of about 2ºC, evidenced by an expansion of the glaciers, among other evidences of an abrupt climate cooling in Europe (VanGeel *et al.* 1996). According to the $\Delta^{13}$C analysis of *P. halepensis* charcoal (Chapter 3), this period appears as a relatively arid phase in the Segre and Cinca Valleys. Such increase in aridity is confirmed by pollen data from El Vilot de Montagut (Alonso *et al.* 2002), and other sites from Catalonia (Riera 1994), as well as hydrological studies from NW Mediterranean (Magny and Richard 1992). However, other palaeoenvironmental proxies provide (apparently) contradictory evidences for this period. Geomorphological studies performed throughout the Ebro Basin (Gutiérrez-Elorza and Peña-Monné 1992) indicated a generalised increase in sediment accumulation on slope deposits around 1000-400 BCE, which was interpreted as an increase in vegetation cover, and a colder/wetter climate. Moreover, pollen analyses from a salt lake in Central Ebro Depresion (Salada Pequeña) suggested an increase in water levels around 2600-2000 BP (650-50 BCE) (Davis 1994). Are these evidences compatible with a water shortage in *P. halepensis* stands?. The answer to this question might come from the role of seasonality. Anthracological evidences from the Cinca and Segre Valleys (Alonso 1999; Ros 1997), show the existence of mixed forests of holm oaks, pines and deciduous oaks, whereas for the Central Ebro Depression the same studies evidenced a decrease in vegetation cover, and increased continentality (Ros 1997). Thus, and according to the adaptive traits discussed in section I, the spread of evergreen and deciduous oaks in the area could be interpreted as a result of an increase in continentality, rather than a consequence of a wetter climate. Although $\delta^{13}$C values in oak from Southern France (Vernet *et al.* 1996), see Fig. 4.3) also indicated an arid episode *ca.* 3000-2700 BP (1050-750 BCE), it was followed by a recovery in $\delta^{13}$C values from *ca.* 2600-1600 BP (650 BCE-350 CE), more than three centuries before the recovery of *P. halepensis* (*ca.* 300 BCE). The same pattern has been recently confirmed by $\delta^{13}$C analysis of oak charcoal (both evergreen and deciduous) recovered from the same archaeological sites included in Chapter 3 (Alonso et al., unpublished results). According to Fig. IXb, such divergence between pines and oaks could be interpreted as an increase in winter precipitation, along with a decrease in spring-summer rainfall. Indeed, $\Delta^{13}$C analyses of cereal grains (see Chapter 6), indicated that water inputs during grain filling (late-spring) were significantly reduced during this period in the area, and also in other sites of NE Spain (Araus *et al.* 1997; Araus and Buxó 1993; Ferrio *et al.* 2005a). On the other hand, as deciduous oaks would be less adapted than evergreen oaks to summer drought, a decrease in summer precipitation would explain the relative increase of the latter reported by Jalut *et al.* (2000). The observed increase in lake levels (as well as glaciers) could be derived exclusively from increased winter recharges. Similarly, a reduction in soil erosion might arise from the absence of convective storms (Gutiérrez-Elorza and Peña-Monné 1992), together with an increase in deep-rooted, cold-resistant species.

## III. Time course of $CO_2$ during the Holocene and the use of carbon isotopes in plant material as palaeoenvironmental tools

According to ice-core data (http://www.ncdc.noaa.gov/paleo/icecore.html) recent changes (i.e. during the last 150 years) in atmospheric $CO_2$ composition exceed those attained in the last half a million years. Thus, we should consider this when using present $\Delta^{13}C$-climate relationships to reconstruct water availability from the archaeological record, as recent increases in $CO_2$ partial pressure might have a direct effect on $\Delta^{13}C$ values. Increasing ambient $CO_2$ ($C_a$ in Eq. IV) tends to decrease stomatal conductance, but also stimulates $CO_2$ assimilation rate through increased substrate supply for carboxylation (Farquhar *et al.* 1982). Bearing in mind that the relationship between water availability and $\Delta^{13}C$ is mostly driven by the stomatal limitation of the intercelular $CO_2$ concentration ($C_i$), significant changes in $C_a$ might alter such relationship. Studies on tree-rings (Bert *et al.* 1997; Duquesnay *et al.* 1998; Saurer *et al.* 2004) and herbarium material (Beerling and Woodward 1993; Friedli *et al.* 1986; Pedicino *et al.* 2002; Peñuelas and Azcón-Bieto 1992) have shown a decrease in $\Delta^{13}C$ from pre-industrial to present times, although this trend varied considerably among species. Thus, for example, in the work of Peñuelas and Azcón-Bieto (1992), the lack of a clear trend for $\Delta^{13}C$ in *P. uncinata* (potentially more sensitive to $C_a$ changes due to its mountain habitat) contrasts with the negative drift of *P. pinea*, and the upward trend of *Alnus glutinosa*. On the other hand, we should bear in mind that, during this period, the raise in $C_a$ comes together with an increase in global temperature, and increased aridity in many areas (Folland *et al.* 2001). Thus, in this kind of retrospective studies, it is difficult to discriminate between $C_a$ and climate effects on $\Delta^{13}C$. Interestingly, experimental studies under controlled environments have shown that $\Delta^{13}C$ of a variety of $C_3$ plants suffer little change over a wide range of $CO_2$ partial pressures (Beerling and Woodward 1995; Picon *et al.* 1996; Picon *et al.* 1997; Polley *et al.* 1993). In most cases, the increase in assimilation rates was compensated by a decrease in stomatal conductance, thus minimising variations in the ratio of intercellular to atmospheric partial pressure of $CO_2$ ($C_i/C_a$) and $\Delta^{13}C$. Moreover, the relationship between stomatal conductance and plant water status is comparable under different $CO_2$ concentrations (Bunce 2004; Picon *et al.* 1996; Picon *et al.* 1997). Overall, these results indicate that $\Delta^{13}C$ can be useful to estimate past water availability, regardless of atmospheric $CO_2$ changes. In addition, the variability of $C_a$ associated to the (unknown) canopy density in which archaeological remains were grown might exceed recent changes in $CO_2$ partial pressure in the atmosphere. This drive our attention to the need of a good amount of replicate samples to avoid an artificial biasing of $\Delta^{13}C$ values (due to variable amounts of refixation of isotopically depleted $CO_2$ from respiratory processes), but also implies that observed differences between archaeological and present material can be hardly attributed to a “$CO_2$ effect”. Nevertheless, the variety of long-term physiological and ecological responses observed in plant systems against $CO_2$ increase still remains to be fully understood (Körner 2003), and thus there is still some degree of uncertainty in past climate inferences based on the analysis of plant material.

## IV. Reconstruction of plant growing conditions in ancient crops: main limitations and future prospects

Overall, the aim of Chapters 4 to 6 was to find alternative approaches to maximise the information about ancient crops that can be derived from the study of archaeobotanical remains. We have shown that combining stable isotope analyses from different species can be useful to find evidences of water management practices. Similarly, the existence of potential genetic changes in cereals can be assessed by relating $\Delta^{13}C$ and grain weight estimates. However, there are still some questions that should be considered, in order to obtain the most reliable picture of ancient crops. It should be noted, for example, that current models to estimate water availability in ancient crops have been developed from modern material, grown under conventional agronomic trials. Thus, the application of such models on archaeological material assumes implicitly that no significant changes in $\Delta^{13}C$ can be derived from recent genetic and/or agronomic changes. Another important topic, specially for grain weight estimations, is the origin and amount of the samples analysed, as discussed below.

### *To what extent are grain weight estimations a reliable source to study changes in potential (i.e. genetic) grain size of ancient crops ?*

Grain weight was probably the earliest genetic trait consciously improved by prehistoric farmers, as already stated in several early studies on agriculture, which drove attention to the traditional selection of the biggest grains for sowing (Eguaras 1988; Wilkinson 1969; Zeven 2000). Unexpectedly, our work suggest that, in the Segre and Cinca valleys, little genetic improvement in grain weight occurred until the last two millennia, but these results should be taken with caution. Firstly, we cannot generalise these findings to other regions. In general, values of grain dimensions from other sites are similar in range to those here reported (Buxó 1997; Colledge 2001; Lone *et al.* 1993; Willcox 2004). However, whereas Willcox (2004) has evidenced an increase in grain size throughout the earliest phases of agriculture in the Middle Euphrates, Buxó (1997) did not found any clear trend in grain dimensions since Early Neolithic to Middle Ages, across a wide range of sites from East Iberian Peninsula. On the other hand, as already mentioned in Chapter 5, another inherent risk of our approach is that, except for the sites of *Margalef* and *Tossal de les Tenalles* (Second Iron Age, *ca.* 200 BCE), carbonised remains used in our study did not came from storage elements, and thus might not be representative of the material harvested by farmers. Thus, in most cases we cannot rule out the possibility that the grains were consciously discarded by them. Nevertheless, the grains from these two sites, recovered from storage jars, are still significanty smaller than current material, and similar in size to those found in other archaeological contexts (domestic fires, cooking ovens, room floors, levels of rubble). Moreover, the $\Delta^{13}C$ of seeds from these sites suggests that the environment in which were grown was even wetter than today, further supporting the existence of a genetic constraint to grain size. Consequently, all indicates that, in the studied area, a considerable change in the genetic potential for grain weight has occurred in the area, although not before that during the last two millennia. Unfortunately, the amount of sample available for this period is too small, and we cannot state definitely the starting point for such change. We are now preparing the analysis of new material from Roman and Middle Age sites (Alonso 2005), in order to obtain a more robust assessment of $\Delta^{13}C$

and grain weight evolution for the last two millennia. One key question that still remains unsolved is whether the Romanisation process already included the implementation of “improved” varieties (i.e. with greater grain size) in this area, or the onset of current landraces is more recent. A complementary source of information for this period might come from documentary studies, e.g. taking advantage of the fact that cereal grains have been traditionally used as standards for weight and length measurements (http://www.metrum.org/measures/metrics.htm).

### *Genotype effects on water availability and $\Delta^{13}C$*

Nowadays, $\Delta^{13}$C is considered as a polygenic trait showing a strong heretability in several crops. In wheat, for example, broad sense heritabilities (proportion of total variance related to genotypes) ranges from 60 to 90% (Araus *et al.* 1998; Ferrio *et al.* 2005b). Similar results have been found in barley (Voltas *et al.* 1999), peanut (Hubick *et al.* 1988), common bean (Ehleringer *et al.* 1991), and many other $C_3$ crops (Hall *et al.* 1990; Hall *et al.* 1994). Thus, although environment is by far the main factor responsive for $\Delta^{13}$C variations in $C_3$ plants, the role of genotype should not be neglected. Although most $\Delta^{13}$C studies have been performed on modern varieties, the few including traditional landraces have shown that they tend to show lower $\Delta^{13}$C values than improved cultivars, mainly due to changes in growing cycle duration (Fischer *et al.* 1998; Zhao *et al.* 2001). Consequently, the genetic differences between archaeological crops and modern reference material might have led to an underestimation of water availability in the past. Changes in phenology, and their effect on yield components and $\Delta^{13}$C, are currently under study, using traditional landraces grown together with modern cultivars. This would provide a more reliable reference value for comparison with ancient crops, as well as to provide clues about the causes for such divergence.

### *Traditional practices and water availability of crops*

Traditional agronomic practices (related with water harvesting, sowing and soil conditions) may provide more water to rainfed crops than modern techniques (Capillon and Séguy 2002; Raupp and König 1996). Thus, opposite to the potential effect of genetic changes, the use of ancient agronomic practices would cause higher water availability (and thus higher $\Delta^{13}$C) in ancient crops. For example, ploughing appear to be a very ancestral practice, well documented in Mesopotamia from the fourth millennium BCE, and probably known in Europe from the end of Neolithic or, at the latest, early Bronze Age, being extensively used during the Iberian period (Civil 1994). Early ploughs were entirely of wood, later including metal shares, causing substantially less soil disturbance than modern techniques of soil preparation. After ploughing, seeds were usually sown by hand to a given deep in the soil Thus, ancient ploughing and sowing practices would probably favour the preservation of soil water (Capillon and Séguy 2002). This is partly confirmed by the results from multifactor field trials performed at the Universitat de Lleida, and including different water regimes, nitrogen supplies, and sowing techniques (Cantero et al., unpublished results). According to this experiment, the use of direct sowing (less aggressive, closer to ancient labour works than standard ploughing) results in significantly higher $\Delta^{13}$C, specially in the most favourable environments. Following these results, we are currently compiling material from similar trials performed in other areas, with the aim to refine current models of water availability.

### *Considering other environmental factors: nutrient availability and temperature*

In Mediterranean environments, rainfed cereal agriculture is not only limited by water shortage, but also by the scarcity of nutrients in the soil (Cantero *et al.* 1995; Corbeels *et al.* 1999; Oscarsom *et al.* 1999; Passioura 2002), and the occurrence of high temperatures during grain filling (Calderini *et al.* 1999a; Calderini *et al.* 1999b; Ouabbou and Paulsen 2000).

Some agricultural practices, such as the addition of organic fertiliser or crop rotation with legumes can leave a characteristic isotope signature in crop plants. As nitrogen isotope composition ($\delta^{15}N$) increases with trophic levels, the addition of animal-derived nitrogen (e.g. manure or plant compost) tend to increase the $\delta^{15}N$ of plants (Bergstrom *et al.* 2002; Choi *et al.* 2003; Riga *et al.* 1971). In contrast, crop rotation with legumes, usually having lower $\delta^{15}N$ due to the fixation of atmospheric nitrogen (Shearer and Kohl 1988; Yoneyama *et al.* 1990), is expected to give lower $\delta^{15}N$ values (Högberg 1997; Riga *et al.* 1971). $\delta^{15}N$ is also affected by water availability and indeed it is often correlated with $\delta^{13}C$ (Handley *et al.* 1999; Heaton 1987). Thus, although the fractionation processes affecting $\delta^{15}N$ in plant matter are complex (Choi *et al.* 2003; Hobbie *et al.* 1999; Högberg 1997), combining $\delta^{13}C$ and $\delta^{15}N$ analyses in archaeological grains offer the potential to provide information about the nutritional status of crops. Currently, we determine routinely $\delta^{15}N$ in archaeological seeds, along with $\delta^{13}C$ analyses. In most cases, archaeological grains have higher $\delta^{15}N$ than modern material, suggesting a systematic use of manure, even in Neolithic sites. However, these conclusions are only temptative, due to the scarcity of reference data regarding $\delta^{15}N$ of plants under traditional crop regimes (i.e. without inorganic fertilisers).

The relationship between temperature and the oxygen isotope composition ($\delta^{18}O$) of precipitation has been well established and is the basis for many palaeoenvironmental studies (see Introduction). In plants, however, we must consider the isotopic enrichment occurring during transpiration, which is specially important under Mediterranean environments (see e.g. Chapter 2). In Chapter 2 we have seen that both $\delta^{13}C$ and $\delta^{18}O$ in wood cellulose of *P. halepensis* are, to some extent, affected by vapour pressure deficit (VPD) and water availability. However, whereas $\delta^{13}C$ is more sensitive to changes in water availability than to VPD, the opposite is the case for $\delta^{18}O$. Bearing in mind that VPD integrates both humidity and temperature information, the combined use of $\delta^{13}C$ and $\delta^{18}O$ would be of great interest in climate reconstructions. Unfortunately, the need for cellulose extraction prevents the application of $\delta^{18}O$ in wood charcoal. The relatively homogeneous nature of cereal grains offer the possibility to be an alternative material for the application of $\delta^{18}O$ isotopes for palaeoenvironmental reconstruction. Indeed, most of the aforesaid considerations regarding $\delta^{13}C$ and $\delta^{18}O$ in cellulose might be valid for cereal grains (Barbour *et al.* 2000; Ferrio *et al.* 2005b; Pande *et al.* 1995). However, the amount of data available about the effect of environmental variables on the $\delta^{18}O$ of cereal grains is still scarce. Some preliminary results for the Mediterranean Region (Fig. X) indicate that combining $\delta^{13}C$ and $\delta^{18}O$ analyses in cereal grains might provide information not only about water availability, but also about temperatures during the growing cycle. Nevertheless, it remains to be tested the effect of carbonisation on $\delta^{18}O$, which can be considerable, due to the important oxygen losses occurring during this process (Kollmann 1955). We are currently performing isotope analyses on cereal grains provided by the GENVCE Spanish winter cereal trial network, in order to study the environmental signal that can be retrieved from the combined use of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$, as well as the effect of carbonisation on $\delta^{18}O$. Moreover, we have determined average grain weight for every sample analysed, and information on grain yield and other agronomic variables is publicly available for these assays. With these data we aim to develop new multi-proxy models to infer crop growing conditions and yield in ancient agriculture.

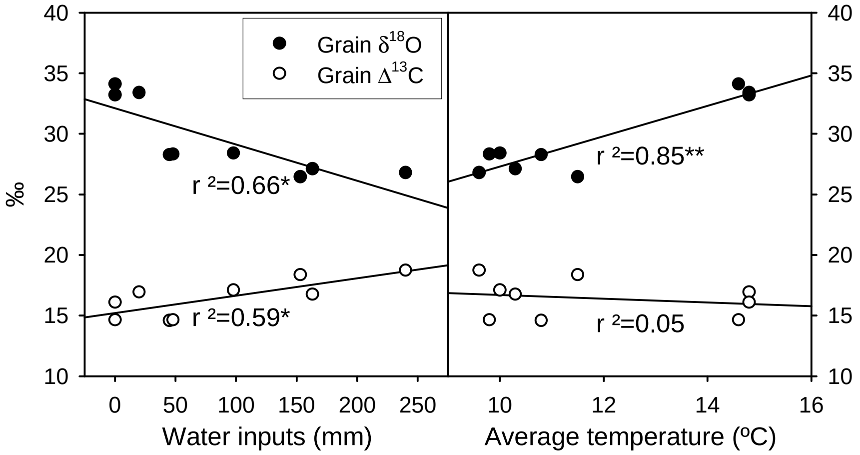


**Fig. X** Relationship between carbon isotope discrimination ($\Delta^{13}C$) and oxygen isotope composition ($\delta^{18}O$) in wheat grains and either total water inputs during grain filling and average temperature throughout the growing season, across nine field trials located in Catalonia (3), Andalusia (3) and NW Syria (3). Data compiled from Araus *et al.* (2003), Ferrio *et al.* (2005b) and Villegas *et al.* (unpublished results). **P*<0.05 ***P*<0.01

## V. Concluding remarks

The application of stable isotope techniques to environmental archaeology is still in its beginnings. Since the pioneer works of Araus and Buxó (1993) and February and Van der Merwe (1992), many advances have been made in the understanding of crop and forest ecophysiology, while the interest of archaeologists in botanical remains has increased notably, to the point that archaeobotanical studies are routinely performed in most archaeological excavations. The ultimate aim of this thesis was to expand the amount of information that can be retrieved from archaeological plant remains. The development of a methodology to obtain reliable estimations of past precipitation regimes from the analysis of wood charcoal might contribute to the knowledge of past climate changes, moreover to being an additional source of environmental data for archaeologists. This methodology would be hopefully expanded to other species, as well as to other areas in the world, where information on past water availability is difficult to attain through other methods. In this context, the EC project MENMED, in which we are currently involved, is giving promising results for the Near East. On the other hand, the required calibration of the climate effects on stable isotopes in extant wood material has also some interest *per se,* as Mediterranean trees are still the “poor brother” of stable isotope studies, and they might help to understand the particular behaviour of these species. Finally, we have proposed some new approaches regarding the reconstruction of the environmental conditions in which ancient crops were grown, such as the objective comparison of data from crop and forest species, as well as from different crops, or the quantification of grain weight. They are promising tools, but are still in a preliminary phase, and surely they will require further refinement. To conclude, the works here presented constitute just another piece in the complex puzzle of the origin and spread of agriculture. In this context, stable isotope techniques can help to obtain a clearer picture of the environmental constraints faced by early farmers. It is unlikely, however, that any technical advance (apart from H.G. Wells’ time-machine) would provide a complete answer for the most intriguing question on the origins of agriculture, that is the reason that pushed hunter-gatherers to initiate such a self-defeating life-style. In words of a Bushman (Lee and DeVore 1968):

*“Why should I farm when there are so many mongongo nuts?”*

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# Conclusions

*“El bambú florece una vez cada cien años,*

*el ciruelo florece una vez al año,*

*el pino permanece siempre verde”*

## I. Reconstruction of climate

### Ecophysiological studies on extant trees

I.1 Aleppo pine (*Pinus halepensis*) is more sensitive to water availability than holm oak (*Quercus ilex*), showing a steeper raise in $\delta^{13}C$ with increasing aridity. This confirms our hypothesis that a partially drought-tolerant species, such as *Q. ilex*, would show lower increase in intrinsic water use efficiency ($WUE_i$) under drier conditions than a drought-avoiding species (e.g. *P. halepensis*).

I.2 $\delta^{18}O$ in holocellulose extracted from *P. halepensis* was mostly associated to variations in vapour pressure deficit, being unrelated to the $\delta^{18}O$ of precipitation. This confirms our hypothesis that, in a species with strong stomatal regulation, the signal of source water ($\delta^{18}O_R$) would be lost due to the great variability in evaporative enrichment at the leaf level.

### Climatic inferences from modern wood

I.3 *Q. ilex* and *P. halepensis* respond to a decrease in water availability by increasing their $WUE_i$, as inferred from the negative relationship between carbon isotope composition ($\delta^{13}C$) in wood and both precipitation and the ratio between precipitation and evapotranspiration. Hence, in these species, $\delta^{13}C$ can be used as an indicator of water availability for (palaeo)environmental studies.

I.4 For *P. halepensis*, $\delta^{13}C$ in whole wood provides similar relationships with climatic variables as those obtained with holocellulose. Indeed, the climatic signature of carbon isotopes is also present in lignin, the other major component of wood. Thus, it is not required to purify holocellulose to use $\delta^{13}C$ of wood from *P. halepensis* as a (palaeo)environmental proxy.

I.5 For *P. halepensis*, holocellulose purification is however necessary to obtain consistent relationships between oxygen isotope composition ($\delta^{18}O$) and climatic variables, at least when working with the usual climatic ranges found in tree-ring studies. This is caused by the presence of a strong variability in lignin $\delta^{18}O$, not associated to climatic variables.

I.6 For *P. halepensis*, $\delta^{18}O$ in holocellulose, as $\delta^{13}C$, is related to precipitation and vapour pressure deficit. However, the information from the two isotopes is complementary, as each one respond in a different manner to these environmental variables, and the two isotopes are poorly correlated.

I.7 The particular adaptive properties at the species level strongly determine the kind of information provided by $\delta^{13}C$ and $\delta^{18}O$. Thus, a careful selection of the material of study (e.g. taking co-occurring species with contrasting physiological performance) would probably increase considerably the environmental information obtained from stable isotopes in tree-rings.

## Stable isotopes in fossil charcoal and palaeoclimate

I.8 Although $\delta^{13}C$ in wood varies considerably after carbonisation, the environmental signal of wood $\delta^{13}C$ is well preserved after carbonisation, and the shifts in $\delta^{13}C$ caused by this process can be successfully removed.

I.9 The study of $\delta^{13}C$ in charcoal from *P. halepensis*, recovered in archaeological sites from the Cinca and Segre Valleys (Ebro Depression), indicate that average precipitation (and the ratio between precipitation and evapotranspiration) was about 30% higher than in present-times from *ca.* 2000 BCE to XVIIIth century. Hence, present-day semi-arid conditions may be mostly due to recent climatic shifts towards a harsher climate, probably enhanced by anthropogenic disturbance.

I.10 There were two main phases of increased precipitation, (1500-900 BCE; 300 BCE-300CE), alternating with phases of lower water availability, relatively closer to current conditions (2000-1500 BCE; 900-300 BCE). A period of strong climatic variability was identified from the tenth to the eleventh century, followed by relatively humid conditions from the sixteenth to the eighteenth century.

I.11 By combining $\delta^{13}C$ analyses from species with different growing cycles and water management strategies, such as *P. halepensis* and *Q. ilex*, it may be feasible to obtain additional climatic information on the seasonality of precipitation events.

## II. Reconstruction of crop conditions

II.1 Naked wheat (*Triticum aestivum/durum*) and hulled barley (*Hordeum vulgare*) were generally grown under better water status in the past than in present-times. This is valid for cereals cultivated in NE Iberian Peninsula (from ca. 5800 BCE to XIth century), SE Iberian Peninsula (*ca.* 4500-300 BCE), as well as in the Fertile Crescent (*ca.* 7900-6500 BCE). In NE Iberian Peninsula, the general agreement between estimations of annual precipitation, derived from fossil charcoal, and estimated water inputs in cereals, suggests that the water status of cereal crops was mostly determined by climatic factors, rather than by agronomic practices.

II.2 In some of the sites from NE and SE Iberian Peninsula, wheat was probably favoured by ancient farmers, sowing it in better soils than barley, or under supplementary irrigation. Our results also indicate that grain legumes (*Vicia faba* and *Lens* sp.), were usually grown under more humid conditions than cereals, most likely in small irrigated plots.

II.3 There is considerable change in dimensions and morphology of wheat and barley grains during carbonisation, but the products Length × Breadth and Length ×Thickness are relatively stable in a wide range of carbonisation environments. From these products (measurable in archaeological grains) it is possible to obtain reliable quantifications of grain weight in ancient cereal crops.

II.4 Within the Cinca and Segre Valleys, grain weight was significantly smaller in the past (from *ca.* 2000 BCE to XIth century) than in present-times. There was an overall trend of increasing grain weight since *ca.* 1000 BCE, becoming steeper from Middle Age to present times. According to $\delta^{13}C$ data, the most recent increase in grain weight was not associated to increased water availability. Thus, the greater grain weight in present-day cereals respond to a genetic improvement of the crops, which might have started sometime from Roman period.

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