Molecular Markers as tools

Applications:
- Productivity
- (Paleo) Environmental conditions
- Temperature
- CO₂ concentration
- Salinity
- Benthic and photic zone oxicity/anoxicity
- Marine vs riverine vs eolian input
- Ecosystem studies
- Climate change
- Paleoclimate ("Molecular stratigraphy").

Molecular stratigraphic tools:
- Paleothermometers (alkenone unsaturation).
- Paleobarometers (alkenone, chlorin δ¹³C).
- Paleochronometers (molecular ¹⁴C).
- Paleotracers (e.g., leaf waxes in dust).

Organic "Paleothermometers":
Long-chain ketones (alkenones) as SST indicators

Reading list
- Proceedings of a workshop on alkenone-based paleoceanographic indicators (www.g-cubed.org, 2000)
The alkenone story - birth of a novel paleoceanographic tool

1978
- Boon et al., 1978
- First identification in sediments
- DSDP core from Walvis Ridge, SW Africa
- Technique: field desorption-MS of total lipid extract and TLC fractions
- Identified as ketones with elemental composition of C_{37}H_{70}O (m/z 530) and C_{38}H_{72}O (m/z 544)

1980
- de Leeuw et al. 1980
- Confirmation of structure as C_{37}-C_{39} methyl and ethyl ketones
- Volkman et al. 1980
- Identification of same compounds in Emiliania huxleyi
- Feeding experiments reveal conservative behavior on passage through gut of zooplankton and excretion as fecal pellets
- Identification of associated compounds (C_{31}-C_{38} odd-chain alkenes) in E. huxleyi
- Formed throughout growth cycle of E. huxleyi
- Proposed as markers for E. hux.
Alkenones – Magical Molecules!

C_{37} “alkenone”

Photomicrograph of E. Huxleyi

Alkenones
- C_{37}Me hexatriaconta-19E,22E-dien-3-one
- C_{37}Me heptatriaconta-8E,15E,22E-trien-2-one
- C_{37}Me heptatriaconta-8E,15E,22E,29E-tetraen-2-one
- C_{37}Me octatriaconta-19E,22E-dien-3-one
- C_{37}Me octatriaconta-9E,16E,23E-trien-3-one
- C_{37}Et octatriaconta-9E,16E,23E-trien-3-one
- C_{37}Et nonatriaconta-17E,24E-dien-3-one
- C_{37}Et nonatriaconta-10E,17E,24E-trien-3-one

Alkyl alkenoates
- C_{37}Me octatriaconta-14E,21E-dienoate
- C_{37}Et octatriaconta-14E,21E-dienoate

Alkenes
- C_{36} heptatriaconta-8E,15E,22E-trien
- C_{36} octatriaconta-9E,16E,23E-trien

Dialkylthiolanes & dialkylthianes
- C_{36}DATL 2-heptadecyl-5-hexadecylthiolane
- C_{36}DATN 2-heptadecyl-6-pentadecylthiolane
Emiliania huxleyi

Affiliation and Evolution
- Class: Haptophyta (Prymnesiophyta)
- Order: Isochrysidales
- Family: Gephyrocapsaceae
- *E. huxleyi* first appeared during late Pleistocene (ca. 250ka)

Distribution and Abundance
- Cosmopolitan eurythermal species (sub-polar to equatorial regions)
- Often found in high concentrations (up to \(5 \times 10^3\) l\(^{-1}\))
- Occasional development of dense blooms
- Most widespread extant coccolithophoric species
- Dominant in transitional and subarctic floral zones
- Isochrysis/Chrysotila limited to coastal environments
- *E. huxleyi* considered to be the dominant source of alkenones in the open ocean
- Predominant in the upper 200m of the water column in the subarctic Pacific ocean
- Constitutes between 40-87% and 40-67% of coccoliths in surface sediments in the North Atlantic and Pacific oceans respectively

Some Definitions:
- **Class**: A taxonomic group containing one or more orders.
- **Order**: A taxonomic group containing one or more families.
- **Family**: A taxonomic group containing one or more genera.
- **Genus (pl. Genera)**: The second most specific taxonomic level, includes closely related species. Interbreeding between organisms within the same genus can occur.
- **Species**: A taxonomic category subordinate to a genus (or subgenus) composed of **individuals** possessing common characteristics distinguishing them from other categories of individuals of the same taxonomic level. In taxonomic nomenclature, species are designated by the genus name followed by a **Latinized** adjective or **noun**.
- A taxonomic group whose members can interbreed.
Emiliania huxleyi

Morphology and Composition
• 2 distinct morphotypes
  • warm water form and cold water form

Alkenone characteristics
• long chain-length (C$_{37}$-C$_{39}$)
• spacing of positions of unsaturation (C-7 not C-2 and C-3)
• double-bond configuration (i.e. E not Z)
• major components of living cell carbon (5-11%)

Other features
• Co-occurring methyl and ethyl alkenoates
• C$_{27}$-C$_{37}$ odd carbon number alkenes
• Carotenoid: 19'-hexanoyloxyfucoxanthin
• Unusual water-soluble acidic polysaccharide
Long-chain ketones

- Recognized in three genera of prymnesiophycean algae
  - *Emiliania*
  - *Chrysotila*
  - *Isochrysis*

**Biosynthesis and biological role**
- Algae biosynthesize alkenones from CO$_2$ via a C$_{36}$ alkenoic acid precursor (Volkman et al., 1980)
- Precise biological role not known
- Believed to be membrane fluidity regulators (lipid bilayer)
  - "margarine vs butter" analogy

**Occurrence**
- Identified in sediments from a wide variety of depositional environments (see table)
- Also identified in freshwater (lacustrine) sediments
- Occur in POM in Atlantic and Pacific oceans
- Found in remote marine aerosols collected on New Zealand (introduced into the atmosphere by bubble bursting - Sicre et al., 1990)

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The alkenone story - birth of a novel paleoceanographic tool

**1984**
- Marlowe et al. 1984
  - Alkenones found to be common to Prymnesiophyceae
  - Alkyl alkenoates found as associated related compounds
  - Chemotaxonomic value confirmed
  - Degree of unsaturation related to growth temperature

**1985**
- Cranwell et al. 1985
  - Alkenones identified in freshwater lake sediments
- Farrimond et al. 1986
  - Alkenones reported in Cretaceous black shales
  - Demonstrates additional biological precursor for alkenones pre-dates appearance of *E. huxleyi*

**1986**
- Brassell et al., 1986
  - Relationship in degree of unsaturation and d18O observed
  - Proposal as a molecular marker for sea-surface temperature
  - Introduction of parameter, Uk37
  - Correlation between latitude, SST and Uk37 in Quaternary sediments
  - Introduction to the concept of *molecular stratigraphy*
Fig. 5. Geological age ranges of genera belonging to the family Gephyrocapsaceae, and the reported sedimentary occurrence of long-chain alkenones. (Modified from Markle et al., 1985). The dotted lines reflect age ranges where alkenones have not been reported from sediments.

Gas chromatograph of TLE of Kane Gap sediments
Latitudinal variations in $U^{137}$ values of sediments and particulate samples
The alkenone story - birth of a novel paleoceanographic tool

1987
Prahl and Wakeham, 1987
• Calibration of Uk37 w.r.t. SST for natural POM populations (sinking and suspended) in Atlantic and Pacific oceans

1988
Prahl et al, 1988
• Calibration of Uk37 vs laboratory cultures of E. huxleyi (commonly accepted calibration)
• Confirm systematic changes in
  • degree of unsaturation
  • overall chain length distribution
  • proportion in alkyl alkenoates/alkenones

Rechka and Maxwell, 1988
• Complete structural assignment of alkenones
• Found to be unusual all $E$ (trans-) configuration
• Refractory nature postulated to be related to unusual double-bond configuration

Prahl & Wakeham, 1988
Alkenone Unsaturation as an Indicator of SST

Fundamental relationship
• A decrease in temperature leads to an increase in the degree of unsaturation
• Initial ratio:
  \[ U_{-37}^{\prime} = \frac{[C37:2]-[C37:4]}{[C37:2]+[C37:3]+[C37:4]} \]
  (Brassell et al., 1986)
• Modified to:
  \[ U_{37}^{\prime} = \frac{[C37:2]}{[C37:2]+[C37:3]} \]
  (Prahl and Wakeham, 1987)
• Ratio can be measured very precisely (GC-FID)

Calibration
• Most commonly used:
  \[ U_{37}^{\prime} = 0.033T + 0.043 \] (Prahl and Wakeham, 1987)
  \[ U_{37}^{\prime} = 0.033T + 0.044 \] (core-top calibration of Muller et al.).
• Accuracy of SST estimation: ± 1°C (in open ocean, temperate and sub-polar waters)

Measurement of Alkenone Unsaturation

Conventional method
• Solvent extraction
• Column chromatography or Thin layer chromatography
• Gas Chromatography

Purification methods
• Silylation
• Transesterification
• Solid phase extraction

Novel detection methods
• Short-column gas chromatography-CI mass spectrometry
• DEI-HRMS (DT-MS)
• GC/TOF-MS
• GCxGC
10 C culture

25 C culture

Washington coast

North Pacific

Prahl et al., 1988

Rechka & Maxwell, 1988
The alkenone story - birth of a novel paleoceanographic tool

- 1989
  - *Poynter et al.* 1989
  - Analysis of "stacked" core records confirmed Uk37 vs δ18O relationship
- 1990
  - *Marlowe et al.* (1990)
  - Micropaleontological and molecular data suggests genera belonging to family Gephyrocapsaceae were all potential sources of alkenones in sediments deposited since Eocene (45Ma). Cretaceous samples - ancestors of this family
  - *McCaffrey et al.* (1990)
  - Alkenone Uk37 found to record short-term climatic variations (El Nino events) in Peru margin sediments over last 300yrs.
  - *Jasper and Hayes* (1990)
  - δ13C of alkenones used for reconstruction of pCO2 over last 70kyr from quaternary sediments (Pigmy basin, Gulf of Mexico) - correspondence with Vostok ice core record.

Molecular stratigraphy of Pigmy Basin sediments, Gulf of Mexico
(Jasper and Gagosian, 1987)
The alkenone story - birth of a novel paleoceanographic tool

1992

Conte et al. (1992)
- Calibration of alkenone and alkyl alkenoate distributions in Eastern North Atlantic (high latitude, cold water).
- Assessment of diagenetic alteration in water column and in sediments indicates SST signature preserved, despite significant compound loss
- Definition of new parameter based on alkyl alkenoate abundance, “AA36”

Kennedy and Brassell, (1992)
- Annual climatic variations over 20th century interpreted from Uk37 in Santa Barbara basin laminated sediments

Freeman and Wakeham, (1992)
- Analysis of Uk37 in Black Sea sediments indicates a different calibration required.
- Different d13C values for C37:4 relative to C37:2 and C37:3 - different sources?

Eglinton et al. (1992)
- High resolution Uk37 record produced through automated sample processing and analysis.

Water column-based SST calibration

![Diagram](Conte et al., 1992)
Alkenone-based SST records of El Nino

Kennedy & Brassell, 1992

Alkenones in the Black Sea
(Freeman and Wakeham, 1992)
The alkenone story - birth of a novel paleoceanographic tool

1993
• Jasper and Hayes, (1993)
• d13C of alkenones used to estimated fraction of marine carbon in Quaternary sediments.
• Rostek et al. (1993)
• Application of coupled Uk37 and d18O records to estimate salinity.
• Sikes and Volkman (1993)
• Extension of Uk37 temperature calibration below 11 deg C.
1995
• Volkman et al. (1995)
• Identification of alkenones in Gephyrocapsa oceanica.
1998
Muller et al. (1998)
• “Global” core top Uk37 calibration.
1999
• Sachs et al. (1999)
• Very high resolution Uk37 record for NW Atlantic across MIS-3.

Reconstructing sea surface temperature and salinity using alkenone and δ18O records

Rostek et al. (1993)
Calibration of alkenone unsaturation ratios for paleotemperature estimation in cold polar waters

Sikes & Volkman (1993)

Alkenones in *Gephyrocapsa oceanica*
Global core-top calibration of $\text{UK}^{37}_{37} \text{ vs SST}$

(Muller et al., 1998)

The alkenone story - birth of a novel paleoceanographic tool

2000
- Benthien and Muller 2000
- Evidence for lateral transport of alkenones.

2001
- Zink et al.
- Temperature relationship observed in alkenones from freshwater lakes

2001
- Xu et al.
- Identification of a novel ($\text{C}_{36:2}$) alkenone in Black Sea sediments

2002
- Ohkouchi et al. (2002)
- Temporal offsets observed between alkenones and planktonic foraminifera in a marine sediment drift.

- The future?
Seasonal variations in depth of alkenone production

Lateral transport of alkenones to the Argentine Basin

Benthien & Muller, 2000
Alkenones in freshwater lakes

Zink et al. 2001

A novel alkenone in Black Sea sapropel (Unit II)
(Xu et al., 2001)
Coupled molecular and microfossil $^{14}$C measurements

Premise:

- Marine algal biomarker compounds (e.g., alkenones) and planktonic forams both encode surface ocean-derived signatures (incl. $^{14}$C content of DIC).
- Age discrepancies must therefore indicate different subsequent fates.
- Marine organic matter is predominantly associated with the fine fraction of sediments – prone to resuspension and redistribution.
- Foraminiferal tests are coarse, sand-sized particles – less susceptible to redistribution by bottom currents.

Approach:

- Use $^{14}$C relationships between planktonic foraminifera, algal biomarkers (e.g., alkenones), and bulk OC isolated from the same sediment intervals as a tool to examine sedimentological processes (lateral transport, bioturbation).
Sedimentological Controls on Geochemical Records from the Bermuda Rise

Ohkouchi et al. 2002
SeaWIFS satellite image of *E. huxleyi* bloom off Newfoundland in the western Atlantic on 21st July, 1999.

Long-range transport of organic matter (and alkenones) from the Scotian Margin to the Bermuda Rise?
Lateral transport of organic matter to the Bermuda Rise

Lateral transport of organic matter to the Bermuda Rise
Sources and Transport of Alkenones and Forams to the Bermuda Rise

Present Day

Little Ice Age

Namibian margin (Benguela Upwelling region)

Data from Mollenhauer et al., 2003
**14C ages of Namibian margin sedimentary components (core 226660-5)**

![Graph showing 14C ages of Namibian margin sedimentary components](image)

Mollenhauer et al., 2003

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**Accumulation maximum on upper continental slope**

![Graph showing accumulation maximum on upper continental slope](image)

Sites of increased accumulation in protected locations (depocenters): Implication of current controlled sedimentation

Seismic data processing: André Janke

Mollenhauer et al., 2002
**14C age discrepancies between organic matter and calcareous microfossils**

- Bermuda Rise
- Namibian Slope
- Chilean Margin
- NW African Slope
- Santa Barbara Basin
- Namibian Shelf
- Scotian Shelf (Emerald Basin)
- New England Shelf (Mud Patch)
- Gulf of Mexico
- Oman Margin
- Indian Ocean

Closed symbols = Alkenone-foram age discrepancy
Open symbols = TOC-foram age discrepancy

**Estimates of advective alkenone contributions**

Assumptions:
- $\Delta^{14}C$ of indigenous alkenones = $\Delta^{14}C$ of forams
- $\Delta^{14}C$ of advected alkenones = -1000 permil (infinite $^{14}C$ age)
Comparison of alkenone and TOC $^{14}$C ages in surficial (<3 cm) marine sediments

Important Remaining Questions

- How are alkenones biosynthesized and what is their physiological role?
- What are the spatial and temporal productivity patterns for alkenone producers?
- Coastal vs. open ocean
- Vertical distribution in the water column
- Time-periods pre-dating *E. hux.*
- What are the reaction pathways by which alkenones are degraded?
- Is the ketone group or the unsaturation the initial site of attack?
- Influences of oxic vs. anoxic conditions.
- Importance of sediment redistribution processes on alkenone/molecular records.
- Lateral advection (drift deposits).
- Differential bioturbation.