Illustration to come

Properties, Chemistry and Analysis of Dyes

The chemical characterisation by HPLC–PDA and HPLC–ESI–MS of unaged and aged fibre samples dyed with sawwort (*Serratula tinctoria* L.)

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Abstract The acid-hydrolysed extracts of freshly dyed reference fibres of sawwort harvested from several different geographical locations were characterised by the use of high-performance liquid chromatography (HPLC) with photodiode array detection (PDA) and coupled with electrospray ionisation mass spectrometric analysis (ESI–MS^n). A related species, *Serratula coronata* L. was also characterised. The flavonols quercetin, 3-*O*-methylquercetin and kaempferol, and the flavones luteolin and apigenin were observed in all samples. Accelerated ageing studies confirmed the sensitivity of the flavonol components to photo-oxidative degradation. The poor lightfastness and small relative proportion of these flavonol components found in the extracts of freshly dyed sawwort limits their use as sawwort 'markers' in historical samples

Introduction

As part of the 'Monitoring of Damage in Historic Tapestries' (MODHT) project,[1] the chemical characterisation of aged and unaged dyed reference fibres was investigated using high-performance liquid chromatography (HPLC) with photodiode array detection (PDA) and with mass spectrometric detection (MS). The biological sources of textile dyes can be determined by the relative quantification of several chemical components in acid-hydrolysed extracts, but photo-oxidation reactions have the potential to alter these components and make unequivocal determinations of biological sources difficult.[2]

The yellow dye component most commonly detected in acid-hydrolysed extracts from historical European textiles is luteolin, a flavone with perhaps the best lightfastness of all the flavonoids found in yellow plant dyes. When luteolin is detected along with a relatively minor amount of apigenin, the dye source is usually reported as weld (*Reseda luteola* L.). Weld was probably the most widely used of all European yellow dye-plants, however, other dye-plants, including sawwort (*Serratula tinctoria* L.) and dyer's greenweed (*Genista tinctoria* L.), were acknowledged substitutes.[3] Although weld and dyer's greenweed are often reported in the analytical results for yellow historical textiles, it is surprising that no analytical evidence for sawwort in such samples has been reported.

In this paper we demonstrate how the flavonoid profile for sawwort, dyed on alum-mordanted wool and aged by light under accelerated conditions, can be mistaken for weld if the HPLC–PDA results for acid-hydrolysed extracts are interpreted based on the detection of only luteolin and apigenin. Furthermore, a

comparison of the acid-hydrolysed extracts from yarns dyed with sawwort harvested from different geographical locations provides additional information on the characteristic flavonoids found in sawwort. The related species, *Serratula coronata* L. was found to contain the same components as sawwort, but with different relative proportions.

Experimental

Mordanting and dyeing

The mordanting and dyeing of the weld (*Reseda tinctoria* L.) samples was performed by colleagues at the University of Manchester Institute of Science and Technology (UMIST) as part of the MODHT project and followed, as closely as possible, medieval dyeing techniques.[4] The wool yarn was mordanted by dissolving alum (0.22 g) in boiling water (750 mL) and immersing the wool in this solution for two hours before wringing out. To dye the wool (15 g), ready-ground weld (7.5 g) and potassium carbonate (0.75 g) were added to boiling water (750 mL); these amounts were scaled down from bulk dyeings. The mordanted, wet wool was immersed in the dye solution and boiled for one hour before being wrung out and rinsed with both cold and hot water.

The sawwort samples used for the accelerated ageing study were prepared at the National Museums of Scotland (NMS). Pre-scoured wool yarn (1.8 g) was boiled in deionised water (100 mL) containing alum (0.03 g) for two hours before being wrung out and left to dry. The dried leaves and stems of sawwort (*Serratula tinctoria* L.) (0.22 g) were ground and added to boiling deionised water (25 mL) containing potassium carbonate (0.03 g). The mordanted wool was wetted under deionised water before being immersed in the hot dye-bath, which was then allowed to cool for one hour. It was then wrung and rinsed with cold water and left to dry.

The sawwort samples from different geographical locations were also prepared at NMS. Pre-scoured wool yarn (1.2 g) was boiled in deionised water (100 mL) containing alum (0.05 g) for two hours before being wrung out and left to dry. This was then divided into three portions for separate dyeings. The first sawwort sample was harvested from the garden of Dominique Cardon in the Lanquedoc-Roussillon region of France (Location 1). The dried leaves, stems and flowers of the sawwort (*Serratula tinctoria* L.) (0.54 g) were ground and added to boiling deionised water (30 mL) containing potassium carbonate (0.02 g). The mordanted wool was wetted under deionised water before being immersed in the hot dye-bath and boiled for one hour. The yarn was left in the dye-bath for a further two hours as it was allowed to cool, wrung and rinsed with cold water and left to dry. The second sawwort sample, harvested from Mount Aigoual, France (Location 2) and the sample of a related species (*Serratula coronata* L.) harvested from Mount Dzungarian, Kazakhstan (Location 3), were prepared in an identical manner to the first.

Accelerated light ageing studies

The ageing experiment was performed with an accelerated ageing chamber in the Scientific Department of the National Gallery, London (designed and manufactured by Complete Lighting Systems, St Albans). The chamber consists of 12 × 20 W fluorescent tubes (Osram L18W/840 Lumilux cool white) giving an exposure of approximately 8000 lux at the middle of the box. The spectral distribution of the fluorescent tubes has been shown to mimic the effects of natural daylight on dyed fibres.[5] Sample holders were produced from acid-free, conservation-quality card and the dyed fibres were threaded across a 3 cm gap in the card to allow each fibre the maximum exposure (each card contained both dyed weld and sawwort samples and an undyed, alum-mordanted 'blank'). A total of 32 cards, including a dark-aged control, were produced for the study lasting 29 weeks. The sampling regime was as follows. For the first week, one sample was

removed at the start and end of each day. In weeks 2 and 3, one sample was removed per day. From weeks 4 to 8, one sample was removed each week, while in weeks 9 to 13, a sample was removed every second week. Finally, in weeks 14 to 29, a sample was removed every four weeks.

Extraction

Following a standard extraction protocol developed for historical textile samples,[6] a small sample of wool (typically 0.5–2.0 mg) was hydrolysed with 2:1:1 (v/v/v) 37% hydrochloric acid: methanol: water (400 mL) in 2 mL glass conical test tubes for precisely 10 minutes at 100 °C. After rapid cooling, each extract was filtered through 5 mm Analytichem polypropylene frits under positive pressure. The test tubes were rinsed with methanol (200 mL) and the combined filtrates dried by rotary vacuum evaporation over a water bath at 40 °C. Dry residues were reconstituted with 1:1 (v/v) methanol:water (200 mL).

High-performance liquid chromatography (HPLC) with photo diode array (PDA) detection

The equipment comprised a Waters 600 gradient pump and a Waters 2996 PDA detector controlled by Waters Empower software, which also processed the data. The column was a reverse-phase Sphereclone (Phenomenex) ODS2, 5 mm particle size, 150 mm × 4.6 mm i.d., regulated by a column oven at 25 ± 1 °C. A sample extract (20 mL) was injected by way of a Rheodyne injector. A tertiary solvent eluent system of 20% (v/v) methanol in water (A), methanol (B) and 5% (w/v) aqueous phosphoric acid (C) was used at a flow rate of 1.2 mL min^{-1} with the following elution programme: 0–3 min isocratic elution 67A:23B:10C; 3–29 min linear gradient elution to 0A:90B:10C; 29–30 min linear gradient elution to 67A:23B:10C. The system was re-equilibrated at the starting eluent composition for 5 min before the next injection. Solvents were sparged using a vacuum in-line degasser. Chromatographed peaks were monitored at 254 nm, although the PDA detector collected all spectral information between 250 and 750 nm. Detector slit and diode widths were 2.4 nm and the response time 1 s. All proportions are reported as peak areas of the HPLC trace monitored at 254 nm.

High-performance liquid chromatography (HPLC) coupled with electrospray ionisation ion trap mass spectrometry (ESI–MS)

Samples were analysed by a high-performance liquid chromatograph connected in series to a UV-visible detector and an electrospray ionisation ion trap mass spectrometer in negative ion mode. The liquid chromatograph (LC) was a Finnigan Mat Spectra System composed of an AS3000 autosampler, a P4000 pump and a V2000 detector. A reverse phase Prodigy (Phenomenex) ODS3 5 mm particle size column, 150 mm × 2.0 mm i.d., was used and the sample extract (20 mL) was injected by an autosampler. A binary solvent eluent system of deionised water (A) and methanol (B) was used at a flow rate of 0.35 mL min^{-1} with the following elution programme: 0–2 min isocratic elution 70A:30B; 2–30 min linear gradient elution to 10A:90B; 30–32 min gradient elution to 70A:30B, then 32–35 min isocratic elution at 70A:30B.

The mass analysis was performed with a Thermoquest Finningan LCQ mass spectrometer controlled by LCQ Navigator software. Parameters for the electrospray ionisation source were: capillary temperature 200 °C, source voltage 4.50 kV, sheath gas (N_2) flow rate 80 arb. units, auxiliary gas (N_2) flow rate 10 arb. units, and capillary voltage –5.00 V. Additional parameters were: tube lens offset –60.00 V, octapole 1 offset 2.75 V, octapole 2 offset 6.00 V, interoctapole lens voltage 20.00 V, and octapole RF amplifier 400.00 Vp-p.

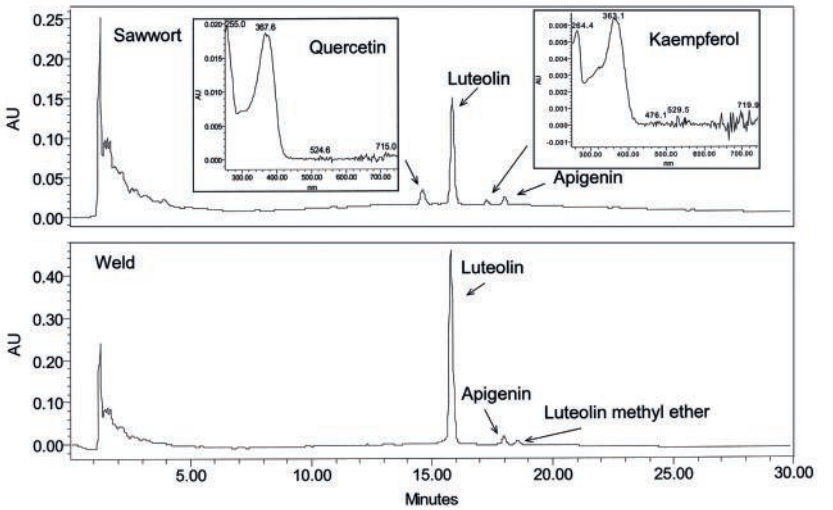

Figure 1 Chromatograms (recorded at 254 nm) obtained from the acid-hydrolysed extracts of alum-mordanted wool reference dyeings of weld (*Reseda luteola* L.) and sawwort (*Serratula tinctoria* L.).

Results and discussion

Unaged weld and sawwort

The chemical characterisation of the acid-hydrolysed extracts was performed using HPLC–PDA and, where further investigative work was required, HPLC–ESI–MS. The chromatograms (at 254 nm) of the freshly dyed samples of weld and sawwort were different (Fig. 1). The main constituent in weld was luteolin (94%), with a minor amount of the flavone apigenin (4%) and a luteolin methyl ether (2%). The constituents are shown in Figure 2 and were identified by matching their retention times and spectral information to an in-house library of standard compounds. The results agree with previously published data.[7]

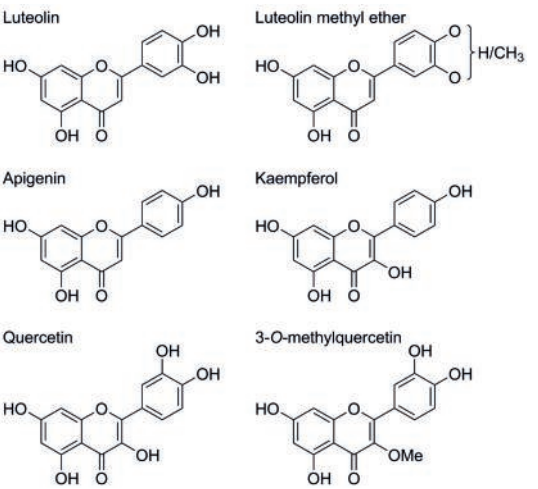

Figure 2 The flavonoid molecules found in weld and sawwort.

The literature on the chemical constituents of sawwort is sparse.[8] One previous paper reported the presence of luteolin and 3-*O*-methylquercetin in the acid-hydrolysed extracts of dyed fibres, using thin layer chromatography (TLC).[9] The presence of other flavonoids in the sawwort plant, including apigenin and kaempferol, has also been reported.[10] The present work on the acid-hydrolysed extracts of alum-mordanted wool, freshly dyed with sawwort, confirms luteolin (78%) as the major component with minor amounts of apigenin (6%) and the flavonol, kaempferol (2%). A second, previously unreported flavonol for sawwort, quercetin

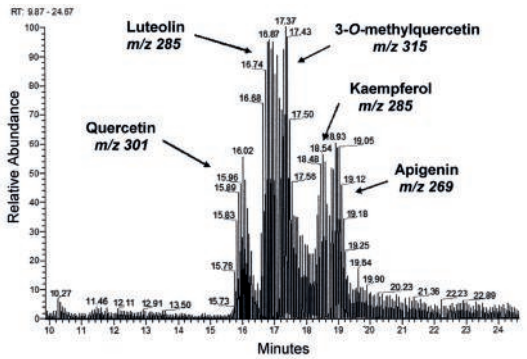

Figure 3 The 'base peak' chromatogram from the ESI–MS, clearly showing the 3-*O*-methylquercetin component co-eluting with luteolin.

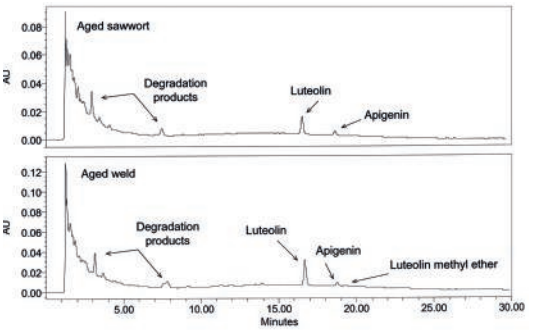

Figure 5 Comparative PDA–HPLC dye profiles (recorded at 254 nm) for sawwort and weld after exposure to accelerated light ageing conditions for 4571 hours.

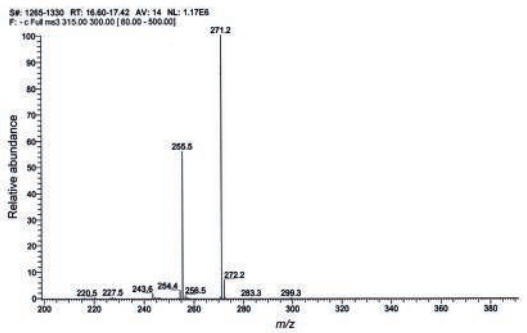

Figure 4 The MS^3 (315, 300) ion trap fragmentation 'fingerprint' for 3-*O*-methylquercetin.

(14%), was also detected. However, the HPLC–PDA results did not indicate the presence of 3-*O*-methylquercetin (see Fig. 2 for chemical structures).

Further investigation of sawwort flavonoid composition using HPLC–ESI–MS^n analysis revealed that a fourth minor component, identified by its $[M\text{-}H]^-$ ion at *m/z* 315 Da, was co-eluting with luteolin (Fig. 3). Tandem mass spectrometric (MS^2) analysis of the ion at *m/z* 315 Da showed a single breakdown peak at 300 Da ($-CH_3$), which suggests replacement of one of the hydroxyl functional groups of quercetin by a methoxy group.[11] Compared with quercetin, the unidentified component has a longer retention time, suggesting greater hydrophobicity, also consistent with the methylation of a hydroxyl group. The MS^3 analysis of the fragment ion at *m/z* 300 from the sawwort sample gave two peaks: *m/z* 271 and *m/z* 255 (Fig. 4). Since the presence of 3-*O*-methylquercetin in sawwort had already been reported, a pure reference sample was purchased (Apin Chemicals Ltd) and analysed under identical conditions. The retention time and breakdown spectra confirmed that the unknown component was indeed 3-*O*-methylquercetin: it had not been detectable by HPLC–PDA because it co-eluted with luteolin.

Accelerated ageing of weld and sawwort

After undergoing accelerated light ageing, the three characteristic components for weld were all detectable by HPLC–PDA (Fig. 5). Their relative proportions after 4571 hours of accelerated ageing were: luteolin (86%), apigenin (11%) and luteolin methyl ether (3%). The relative proportions of the sawwort components after accelerated ageing, under identical conditions and for the same number of hours were: luteolin (78%), apigenin (17%), quercetin (4%) and kaempferol (1%). The differences in relative proportions of the flavonoids for sawwort after ageing can be explained by the preferential degradation of flavonols (quercetin

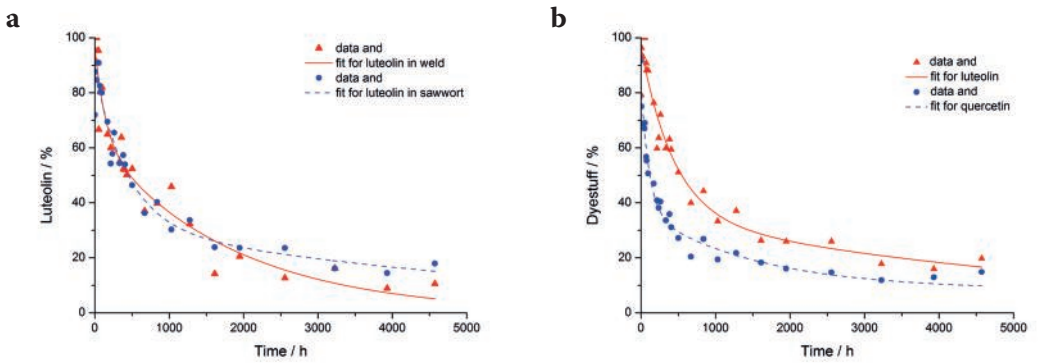

Figure 6 (a) The normalised luteolin signal (peak area/sample mass) for weld and sawwort plotted as a percentage of the maximum luteolin signal against exposure time in the accelerated ageing chamber. (b) The normalised luteolin and quercetin signals (peak area/sample mass) for sawwort plotted as a percentage of the maximum dyestuff signal against exposure time in the accelerated ageing chamber.

and kaempferol) over flavones (luteolin and apigenin) due to photo-oxidation during light ageing.[12]

As expected, the photo-oxidation of the minor flavonol components in sawwort (quercetin and kaempferol) under accelerated ageing conditions proceeded at a faster rate than the flavone components such as luteolin. The luteolin component in both weld and sawwort degrades at the same rate (Fig. 6a), while the quercetin component of sawwort degrades faster (Fig. 6b). As has been indicated previously,[13] the decay of quercetin can be fitted to a double exponential model. This can most easily be interpreted (to a first approximation) as the two different degradation rates associated with the dye on the surface of the fibre and the dye on the bulk of the wool fibre.

The photo-oxidation products of flavonols can be characteristic of the original compound.[14] In cases where flavonols are the minor constituents of a dye source, as for sawwort, they can often fall to levels below the detection limit. Although the photo-oxidation products of flavonols were not detected in sawwort, other degradation products were observed in both the sawwort and weld samples exposed for the maximum time period under accelerated ageing conditions. Two of these are believed to be wool degradation products as they also appeared in the aged, undyed alum mordanted wool sample, while another has yet to be characterised.

In the case of the 3-*O*-methylquercetin, it is likely that the methoxy group at the 3-position would have slowed the characteristic photo-oxidative mechanism of the 3-hydroxy analogues.[15] However, the 3-*O*-methylquercetin appears to have degraded within the timeframe of this experiment, as it could not be detected in the final sample subjected to accelerated ageing conditions. The 3-*O*-methylquercetin was a very minor component in this particular sawwort source and its presence at the start of the ageing regime (but not at the end) is unlikely to have distorted the luteolin degradation results significantly within the error limits of the study. The analysis of the acid extracts from the fibres dyed with weld or sawwort and aged in the accelerated ageing chamber for the full 29 weeks therefore produced remarkably similar HPLC–PDA chromatograms.

Due to the poor lightfastness of the flavonol components in sawwort, it appears that when analysing historical fibres, the detection of a trace amount of luteolin methyl ether with luteolin and apigenin (such as in the example of the herald's tabard)[16] is very important for the assignment of weld as the dye source rather than sawwort.

Table 1 The relative percentages (by peak area at 254 nm) of the five main components in the acid-hydrolysed extracts of *Serratula* species from different geographical locations.

Serratula species and location	Quercetin area (%)	Luteolin + 3-*O*-methylquercetin area (%)	Kaempferol area (%)	Apigenin area (%)
Serratula tinctoria L.1: Lanquedoc-Roussillon	8.0	76.2	3.0	12.8
Serratula tinctoria L.2: Mount Aigoual	6.0	89.0	0.3	4.7
Serratula coronata L. 3: Mount Dzungarian	12.8	75.4	4.1	7.7

Note: Luteolin and 3-*O*-methylquercetin have been calculated together.

Sawwort harvested from two different geographical locations and a related species, Serratula coronata L.

The sawwort used to dye the yarns for the accelerated ageing study was the only authentic sample available to us when this experiment was conducted. However, a comparative study has now also been undertaken with wool yarns dyed with a further two authentic sawwort samples, harvested from different geographical locations, and a third wool sample dyed with a related *Serratula* species. The analysis of the acid-hydrolysed extracts of these samples has allowed a more complete characterisation of the flavonoid composition for sawwort.

Analysis of the extracts by HPLC–PDA and HPLC–ESI–MS found that in each case, the five previously identified components were observed: quercetin, 3-*O*-methylquercetin, luteolin, kaempferol and apigenin. However, these components were observed at different relative amounts in each case (Table 1). The luteolin and 3-*O*-methylquercetin were calculated together as they co-eluted in the HPLC–PDA method. Close examination of the PDA results for the luteolin + 3-*O*-methylquercetin peak in the *Serratula coronata* L. sample suggested that it contained a much greater amount of 3-*O*-methylquercetin relative to luteolin than the sawwort extracts from either Location 1 (Lanquedoc-Roussillon region, France) or Location 2 (Mount Aigoual, France). This was confirmed by HPLC–ESI–MS. The extracts from all locations also contained trace amounts of unidentified colouring components. These were barely above the detection limit of the HPLC–PDA instrument and are therefore not suitable for identifying the use of sawwort in historical samples.

Conclusion

The acid-hydrolysed extracts of reference wool yarns freshly dyed with sawwort harvested from several different geographical locations were characterised by the use of HPLC–PDA and HPLC–ESI–MS^n analysis. The flavonoids of a related species, *Serratula coronata* L. were also characterised. The extracts were shown to contain the flavonols quercetin, 3-*O*-methylquercetin and kaempferol, and the flavones luteolin and apigenin. The HPLC–PDA elution programme used for this analysis did not separate the luteolin and 3-*O*-methylquercetin components. Refining the elution programme would enable separation of these flavonoids. However, the use of HPLC–ESI–MS^n confirmed the presence of both compounds.

Accelerated ageing studies indicate that the detection of luteolin and apigenin together in acid-hydrolysed historical dyed wools is not exclusively indicative of weld, but may also be photo-oxidised sawwort. The poor lightfastness and small relative proportion of the flavonol components found in the extracts of freshly dyed sawwort limits their use as sawwort 'markers' in historical samples. The importance of the detection of the luteolin methyl ether for the assignment of weld has also been highlighted.

Acknowledgements

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Editor's note

Paper received 26 July 2004; revised version 24 February 2005. This paper was presented at *Dyes in History and Archaeology* 22, Riggisberg, 23–24 October 2003. Sadly Hamish McNab died in 2010. At the time of carrying out the work described in this paper, David A. Peggie was at the School of Chemistry, University of Edinburgh and Conservation & Analytical Research, National Museums of Scotland, Edinburgh; Anita Quye was in Conservation & Analytical Research, National Museums of Scotland, Edinburgh.

Chemicals and suppliers

- Weld: Verfmolen De Kat, The Netherlands.
- Sawwort for the accelerated ageing study: Michel Garcia, Association Garance, Lauris, France.
- Sawwort from different geographical locations and *Serratula coronata* L.: kindly harvested by Dominique Cardon.
- 3-*O*-methylquercetin reference: Apin Chemicals Ltd (https://www.google.co.uk/#q=Apin+Chemicals+Ltd)

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11. Ferreira *et al.* 2002 (see note 2 above).
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