#### Pollen Analysis from Lanton Quarry, Northumberland.



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#### **EXECUTIVE SUMMARY**

In December 2006 Archaeological Research Services Ltd (ARS Ltd) were commissioned by Tarmac Northern Ltd to undertake pollen analysis from sediment taken from Lanton Quarry, Northumberland, UK. Three sediment cores were collected from the floodplain adjacent to the Lanton quarry and twelve sediment samples were removed from the core Lan (2).

The cores were collected from the modern floodplain sedimentary sequences that contained organic and inorganic material and retained for palaeoecological analysis. In total twelve sediment samples were processed using an acid digestion with an added density separation stage to enhance pollen concentrations.

The processed samples contain abundant arboreal, herbaceous and aquatic pollen and non-pollen palynomorphs. The range of pollen and non-pollen palynomorphs includes Alnus glutinosa (alder), Pinus (pine), Poaceae (grasses), Avena/Triticum-type (oat/wheat), Myriophyllum alterniflorum (whorled water-milfoil), Rumex acetosella/acetosa (common sorrel/sheeps sorrel), Plantago lanceolata (ribwort plantain) and Filicales (ferns). The preservation of the pollen is predominantly "good" although frequent damaged grains have been recorded. The interpretation of the pollen describes an area dominated by alder with partially cleared or limited mixed woodland but with areas of herbaceous rich grasslands and rough pasture.

The evidence for human activity indicates that pastoral agriculture was widespread. However, there is very limited evidence to suggest arable cultivation occurred within the area or in relatively close proximity to the site. Four organic macrofossils were sent for radiocarbon dating and returned a minimum/maximum date of 2680 - 90 cal. BC for the sedimentary sequence. Cereal cultivation was dated to the Late Bronze Age c.1130 - 900 cal. BC, whilst the fourth date provided further evidence of enhanced fluvial activity within the floodplain system. However, based on pollen and radiocarbon evidence, the oldest sedimentary sequence dates to the Late Neolithic.

#### 1. INTRODUCTION

#### 1.1 Location and Scope of Work

Archaeological Research Services Ltd was commissioned by Tarmac Northern Ltd to undertake pollen analysis at Lanton Quarry, Northumberland. In December 2006 three sediment cores were removed from the site totaling 2.5 m of undisturbed sediment. Pollen analysis was undertaken during February 2007.

#### 1.2. Site Location.

The Lanton Quarry site lies in the Milfield Basin north east of the Cheviot Hills and approximately eight miles north of Wooler. The core site was located (NT 95613050) on the modern floodplain *c*.650 m distant from the south western edge of the Lanton Quarry site (see Fig 1).

#### 1.3. Geology

The underlying bedrock dates to the Carboniferious period overlain by extensive Devensian fluvio-glacial sand and gravel deposits. Over lying the fluvio-glacial deposits are fine grained alluvial deposits that underlie the Holocene floodplain. The sediment core Lan (2) analysed for pollen was located on the Holocene alluvial floodplain.

Figure 1. Site location.



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#### 2.0 Methods

#### 2.1 Core Collection.

Three cores were extracted (Lan(2) 0-0.9m, Lan(2) 0.5-1.5m and Lan(2) 1-2m) on site for environmental analysis, using a Stitz Vibrocorer. The cores were extruded into plastic tubing and wrapped with plastic sheeting in the field and returned to cold storage at Newcastle University. The cores were cleaned, digitally photographed and stratigraphically logged by ARS Ltd under clean laboratory conditions. From the three collected cores one, Lan (2) 0.5–1.5m, was sub-sampled and twelve samples were prepared for pollen analysis. The sample number and depth of the sample (down core) can be seen in Table 1.

Laboratory	Depth of
sample code	sample (mm)
P1	745-750
P2	65-70
P3	135-140
P4	475-480
P5	675-680
P6	275-280
P7	345-350
P8	615-620
Р9	415-420
P10	545-550
P11	205-210
P12	815-820

Table 1. Levels selected for analysis Lan (2) 0.5-1.5

The sampling interval was not uniform but varied throughout the sedimentary sequence with a sample thickness of 5mm employed. At each selected level 1g of sediment was used per sample. Two Lycopodium tablet (batch number 483216) were added to each sample prior to chemical preparation for the purposes of calculating pollen concentrations as described by Stockmarr (1971). The chemical preparation of the samples followed the acid digestion (based on the procedure as described by Barber 1976), with an added density separation stage to concentrate the pollen, which followed the J.J. Lowe and N. Branch (unpublished) Royal Holloway & Bedford New College, University of London method. Further details of the laboratory procedure are contained in Appendix B. All counts were undertaken using a Leica DME compound microscope at a magnification of x400. A standard count of 500 pollen palynomorphs plus nonpollen-palynomorphs was employed. The count included exotic grains (Lycopodium), spores and aquatics to give an indication of pollen concentrations and potential vegetation composition for each level. Identification of pollen grains and spores was aided by the use of published identification keys, including Faegri & Iversen (1989), Moore, Webb & Collinson (1991), Hans-Jürgen Beug (2004) and by comparison with pollen reference material (type slides) held by ARS Ltd.

#### 2.2. Photography

The cleaned cores were placed on a mechanical stage, underneath a fixed position digital SLR camera. The cores were methodically advanced in regular intervals beneath the camera, with high-resolution digital images being taken every 5cm. The images were then combined using ArcSoft Panorama 3.0 software, producing a continuous digital image of each core (Appendix C – attached CD).

#### 2.3 Radiocarbon dating

A total of four levels were chosen for radiocarbon dating. Two samples from the uppermost and basal sedimentary units were removed to establish a min/max age range for the core (Lan06 2A (1) and Lan06 2B (1) respectively). Whilst two (Lan06 2A (2) and Lan06 2A (3)) were chosen from the core based on preliminary pollen analysis results. The samples were sent to Beta Analytic Inc, Florida USA. The sample information is shown in table 2.1.

Sample	Depth (down	Wet weight	Material sent for
identification	core)	sub sampled (g)	radiocarbon dating
	mm		
Lan06 2A (1)	330 -340 (Lan	2.7	Indeterminate twig
	2 0-0.9m)		_
Lan06 2B (1)	737-747 (Lan2	1.2	Alnus macrofossil
	0.5-1.5m)		
Lan06 2A (2)	470-475 (Lan2	2.0	Alnus Roundwood
0.5-1m	0.5-1.5m)		
Lan06 2A (3)	210-215 (Lan2	2.0	Indeterminate twig
0.5-1m	0.5-1.5m)		_

Table 2 Depth of Radiocarbon samples taken from Lan06.

The calibrations of these results, relating the radiocarbon measurements directly to calendar dates, have been calculated using the calibration curve of Reimer *et al* (2004) and the computer program OxCal (v3.10) (Bronk Ramsey 1995; 1998; 2001).

#### 3.0 Results

#### 3.1 Sediment Cores

The cores were extracted from two parallel boreholes, with a distance of 0.2m between the holes. This allows an overlap between the individual cores to be collected, and therefore producing a near continuous undisturbed sedimentary sequence. The parallel boreholes were sunk to *c*. 2.5m depth, and three cores were collected.

#### 3.2. Core Stratigraphy

The individual cores were described and stratigraphically logged and are presented in Figures 2 - 4.

#### Figure 2. Photographic and stratigraphic log of Lan (2) 0-0.9m



Lanton Core 2 Sediment Log 0.5 - 1.5 m	<ul> <li>7.5 YR 2.5/2 Very dark brown homogeneous clay with fine sand.</li> <li>Fine sand content c. &lt;5%, some medium sand &lt;1% but clearly present.</li> <li>Occurence of Fe staining but not laterally extensive. No apparent structure very plastic, easy to deform. Gradational boundary.</li> </ul>	<ul> <li>7.5 YR 2.5/1 Black homogeneous silt with medium sand.</li> <li>Medium sand content &lt;1% but clearly present. No apparent structure Gradational boundary.</li> </ul>	7.5 YR 2.5/2 Very dark brown clay with silts. Fine sand content c. <1.5%. Occurence of organic material but not extensive. No apparent structure. Gradational boundary.	7.5 YR 2.5/2 Very dark brown clay with organics. Occurence of black nodules <2mm possibly manganese staining or larger organics. No apparent structure, no sand. Gradational boundary.	7.5 YR 2.5/1 Black clay with silt and organics. No sand noted, organic remains increasing in size down unit, but appears randon and not sorted. No apparent structure Gradational boundary.	7.5 YR 2.5/2 Very dark brown clay with silts. Fine sand content c. <1.5%. Occurence of organic material but not extensive. No apparent structure. Gradational boundary.	7 10 YR 2/2 Very dark brown clay with silts and organics. Fine sand content c. <3 %. Occurence of organic material and large macrofossil <15 mm. No apparent structure. Gradational boundary.	7.5 YR 2.5/1 Black clay with silt and fine to medium sand. Organics are present with large macrofossil. Sand material has no apparent structure and appears poorly sorted. Sharp boundary.	10Y 3/2 Very dark grayish brown clay with silts, organics and medium to fine sand. Large macrofossils noted. Sand appears to have no apparant structure, poorly sorted but does become finer down unit. Sharp boundary.	10YR 3/1 Very dark gray silt with clay and organics and fine sand (<1.5%). Large macrofossils noted at base of unit. Black nodules seen throughout, possibly manganese staining or decomposed organics. No apparant structure, poorly sorted. Sharp boundary.
		2) (b) (c) (c) (c) (c) (c) (c) (c) (c) (c) (c	(C)		6			• • •		F
	0.04	0.08 0.11 0.13	0.17	0.23 0.25			0.42		0.61	

Figure 3. Photographic and stratigraphic log of Lan (2) 0.5–1.5m



10YR 3/2 Very dark grayish brown silt with organics and large macrofossil (wood) some fine sand but trace amount only.increaseing across boundary with lower unit. No apparant structure. Black nodules possibly manganiese staining or very degraded organics. Gradational boundary.





Figure 4. Photographic and stratigraphic log of Lan (2) 1- 2m

#### 3.3. Pollen Analysis

The results of the pollen analysis have been presented as percentage diagrams using the specialist software TGView (Grimm 1987). The pollen data has been presented as percentages of Total Land Pollen (TLP), excluding Non-pollen-palynomorphs (NPP) and aquatics, which have been expressed as percentages.

The pollen data was placed into local pollen assemblage zones using the statistical technique of CONISS (stratigraphically constrained cluster analysis using sum squared and Euclidean squared distances). Four local pollen assemblage zones (LPAZ) have been identified and unless cited all depths used in the pollen description are for Figure 3.4. For clarity the Lan (2) 0.5-1.5m core shall henceforth be referred to as Lanton Quarry.





#### 3.3.1. Local Pollen Assemblage Zone (LPAZ) 1 610-400mm

The arboreal pollen is characterised by the dominance of *Alnus glutinosa* throughout the zone, followed by *Tilia*. However, by the end of the zone, *Tilia* has been superseded by *Quercus*. Initially *Alnus glutinosa* is in decline (from 40%TLP to 36% TLP) concurrent with an increase in *Corylus avellana*-type (from 16%TLP to 31%TLP) until 470mm depth. At 470mm depth a reversal occurs and *Alnus glutinosa* increases to finish the zone recording 47%TLP whilst *Corylus avellana*-type declines to *c*. 17%TLP. *Tilia* records a two stage decline, the first of *c*. 5% TLP between 620 to 540mm depth and then briefly recovers (from 10%TLP to 16%TLP) until 470mm depth where the second decline begins with *Tilia* recording <5%TLP by the end of the zone. *Quercus* initially increases by *c*. 1%TLP until 540mm depth. From 540 mm depth *Quercus* declines (from 6%TLP to *c*. 2.5%TLP) until 470mm depth and recovers to 5%TLP by the end of the zone. *Pinus* and *Betula* both decline throughout the zone (4%TLP to 3%TLP and 3%TLP <1%TLP respectively) with *Betula* only recording a trace presence by the end of the zone. *Ulmus, Salix, Fraxinus* and *Carpinus betula* all record a trace presence (<1%TLP) through the zone.

The herbaceous types are relatively well represented throughout the zone. Poaceae (8%TLP) is the dominant herb and fluctuates by +/- *c*. 1%TLP until 470mm depth where it records an increase and attains a zone maximum of 13%TLP. The increase is concurrent with the decline in *Corylus avellana*-type. Cyperaceae (*c*.7%TLP) also fluctuates (+/- 1%TLP) until 470mm depth where it increases to 8%TLP, possibly in response to the decline in *Corylus avellana*-type. Human associated types such as *Plantago lanceolata*, *Rumex acetosa/ acetosella*, Ranunculaceae and *Avena/Triticum*-type are present (<1%TLP to 1%TLP) from 54mm depth. Aquatic types such as *Myriophyllum verticillatum* and Haloragaceae are recorded as a trace presence only.

The non-pollen-palynomorphs are relatively well represented. *Filicales* dominates (c. 5.5%) followed by *Polypodium* (<1%). NPP type 36, 114 and 190 are all recorded in trace presence to 1% values. Microscopic <50 $\mu$ m charcoal concentrations decline from 10 cm<sup>2</sup>/cm<sup>3</sup> to *c*. <1 cm<sup>2</sup>/cm<sup>3</sup> at 470mm before increasing to 13 cm<sup>2</sup>/cm<sup>3</sup> by the end of the zone. Microscopic >50 $\mu$ m charcoal concentrations decline from 5 cm<sup>2</sup>/cm<sup>3</sup> and become absent from the zone at 470mm depth.

#### 3.3.2 LPAZ 2 400 - 300mm

Alnus glutinosa increases throughout the zone (from 47%TLP to 55%TLP) and remains the dominant pollen. *Quercus* (5%TLP), *Tilia* (3%TLP) and *Pinus* (3%TLP) remain constant through the zone. *Corylus avellana*-type increases by *c*. 1%TLP until 340mm depth, then decreases for the remainder of the zone. *Salix*, and initially *Betula*, record a trace presence only (<1%TLP). However from 340mm depth *Betula* becomes consistently present in the zone. *Pinus* and *Quercus* are consistently present throughout the zone (3%TLP and 9%TLP respectively) whilst *Tilia* declines (from *c*. 3%TLP to 1.5% TLP).

The herbaceous types are relatively well represented throughout the zone. Poaceae followed by Cyperaceae are the dominant herbs but both are in decline through the initial part of the zone (from 13%TLP to 10%TLP and 6%TLP to 4%TLP respectively). At 340mm depth, a change occurs and Poaceae and Cyperaceae increase (10% TLP to 13%TLP and 4%TLP to c.5%TLP respectively). Additional herbs such as Apiaceae,

*Caltha palustris*, *Potentillia* and *Plantago coronopus* are recorded in trace presence only. Human associated pollen types such as *Plantago lanceolata*, *Avena/Triticum*-type and Ranunculaceae are initially present, but decline to absence by the end of the zone. The aquatic type *Myriophyllum verticillatum* increases (1%TLP to c. 2%TLP) through the zone until 340mm depth then declines for the remainder of the zone.

The non-pollen-palynomorphs are relatively well represented. *Filicales,* followed by *Polypodium* dominate (c. 5.5% and *c*.2%) whilst NPP 114 increases to (c.2%). Microscopic  $<50\mu$ m charcoal concentrations increase (12 cm<sup>2</sup>/cm<sup>3</sup> to 26 cm2/cm<sup>3</sup>) until 340mm depth, whilst  $>50\mu$ m concentrations increase from *c*. 1 cm<sup>2</sup>/cm<sup>3</sup> to *c*. 2.5 cm<sup>2</sup>/cm<sup>3</sup>).

#### 3.3.3 LPAZ 3 300 - 100mm

The arboreal pollen is characterised by the dominance and consistent presence of *Alnus* glutinosa (55% TLP) throughout the first half of the zone. From 200mm depth *Alnus* glutinosa increases (from 55% TLP to c. 57% TLP) until c. 130mm depth where a notable decline occurs and *Alnus glutinosa* ends the zone at c.44% TLP. *Quercus* remains consistent (5% TLP) through the zone until 130 mm depth, and then begins to decline and finishes the zone at <4% TLP. *Betula* increases (from 1% TLP to c. 2% TLP) until 200mm depth where a decline occurs. *Pinus* declines and becomes absent at 200 mm depth, but reappears in the zone at 130mm depth. *Ulmus*, *Tilia*, *Salix* and *Fraxinus* record a trace presence at 200mm and 130mm depth only.

*Corylus avellana*-type declines through the initial part of the zone from 15%TLP to 11%TLP. A change occurs at 200mm depth and *Corylus avellana*-type recovers to 13%TLP until 130mm where a secondary decline (to 10%TLP) occurs for the remainder of the zone.

The herbaceous types are relatively well represented throughout the zones. Poaceae, followed by Cyperaceae, are the most frequently recorded herbs. Poaceae increases consistently (from 10%TLP to 17%TLP) whilst Cyperaceae declines (from 6%TLP to 2% TLP) until 130mm depth. From 130mm depth both Poaceae and Cyperaceae display increases for the remainder of the zone (from 17% to 21% and 2%TLP to *c*.7%TLP respectively). The aquatic type *Myriophyllum verticillatum* declines through the initial part of the zone and becomes absent by 200mm. Pollen types such as Apiaceae, *Urtica, Rhinanthus*-type, Lactuceae and *Cirsium* all record a trace presence. Human associated types such as *Avena/Triticum*-type, *Plantago lanceolata, Linum catharticum, Rumex acetosa/ acetosella* and Ranunculaceae become briefly present in the zone at 200mm depth.

The non-pollen-palynomorphs are relatively well represented. *Filicales,* followed by *Polypodium,* initially dominate, but both decline throughout the zone, with *Polypodium* becoming absent by 200mm depth. NPP type 114 increases and is almost consistently present, following a brief absence at 200 mm depth. From *c*.130mm depth *Osmunda regalis* becomes established in the assemblage. Microscopic charcoal concentrations  $<50\mu$ m decrease (26 cm<sup>2</sup>/cm<sup>3</sup> to 6 cm2/cm<sup>3</sup>) whilst microscopic charcoal concentrations  $>50\mu$ m increase from *c.* 1 cm<sup>2</sup>/cm<sup>3</sup> to *c.* 2.5 cm<sup>2</sup>/cm<sup>3</sup> until 200 mm depth then decline for the remainder of the zone.

#### 3.3.4 LPAZ 4 100 - 0mm

Alnus glutinosa declines throughout the zone (from 47%TLP to 30%TLP) but remains the dominant arboreal pollen. Quercus is in decline throughout the zone (from <4%TLP to <2%TLP). Pinus and Betula increase (from 1%TLP to c. 2%TLP and 1%TLP to c. 2.5%TLP respectively). Ulmus, Salix, Carpinus betula and Tilia all record a trace presence between 60 – 0mm depth. Corylus avellana-type declines from 11%TLP to 10%TLP whilst Lonicera and Euonymus europaeus record a trace presence between 6 – 0mm depth.

The herbaceous types are relatively well represented throughout the zones. Poaceae, followed by Cyperaceae, are the most frequently recorded herbs. Poaceae and Cyperaceae increase from 21%TLP to 27%TLP and c.7%TLP to 10%TLP resp[ectivley. Rosaceae and *Artemisia* increase (from <1%TLP to 3%TLP) whilst *Filipendula* records a trace presence. Human associated types include *Avena/Triticum*-type, *Plantago lanceolata* and Ranunculaceae record trace presence only whilst *Rumex acetosa/acetosella* increases from <1%TLP to c.2.5%TLP.

The non-pollen-palynomorphs are relatively well represented. *Filicales* followed by *Osmunda regalis* dominate. Non-pollen-palynomorphs type 114 increases (from 1% to 2.5%), whilst *Polypodium*, Type 128, 190 and *Sphagnum* records a trace presence only. Microscopic charcoal concentrations  $<50\mu$ m decrease (from 10 cm<sup>2</sup>/cm<sup>3</sup> to 5 cm2/cm<sup>3</sup>) whilst microscopic charcoal concentrations  $>50\mu$ m decrease from *c*. 1 cm<sup>2</sup>/cm<sup>3</sup> to <1 cm<sup>2</sup>/cm<sup>3</sup>).

#### 3.4. Radiocarbon Dating.

A total of four samples were submitted for radiocarbon dating by Accelerator Mass Spectrometry (AMS) at Beta Analytic Inc, Florida, USA. The four samples consisted of two identified *Alnus* macrofossils (LAN06 2A (2) and Lan06 2B (1)) and two indeterminate twig macrofossils (LAN06 2A (3) and LAN06 2A (1)). The four samples provided plenty of carbon for accurate measurement and all the analyses proceeded normally.

The results given in Table 3, are conventional radiocarbon ages (Stuiver and Polach 1977), and are quoted in accordance with the international standard known as the Trondheim convention (Stuiver and Kra 1986). The graphical distributions of the calibrated dates, given in Figure 6, are derived from the probability method (Stuiver and Reimer 1993) using the program OxCal v3.10 (http://units.ox.ac.uk/departments/rlaha).

Lab number	Sample	Material and depth	δ <sup>13</sup> C	C14 age BP	Cal. BP	Calibrated date	Stuiver and Reimer (95%
			$(^{0}/_{00})$			(Intercept	probability)cal. BC/AD
						95%) cal. BC	
Beta-230046	LAN06 2A(1)	Indeterminate Twig	-28	$2160 \pm 40$	2310-2040	360-90 BC	cal. 370 - 90 BC (95.4%)
		33-34 cm					
Beta-230047	LAN06 2A(2)	Alnus roundwood	-29.7	$2840 \pm 40$	3070-2860	1120-910 BC	cal. 1130 –900 BC (95.4%)
		47-47.5 cm					
Beta-230048	LAN06 2A(3)	Indeterminate Twig	-28.6	$3700 \pm 50$	4220-4210	2260-1950 BC	cal. 2280 - 2250 BC (1.7%)
		21-21.5 cm			4160-3900		cal. 2210 - 1940 BC (93.7%)
Beta-230049	LAN06 2B(1)	Alnus macrofossil	-28.4	$4010 \pm 50$	4780-4770	2830-2460 BC	cal. 2850-2810 BC (2.9%)
		73.7 -74.7 cm			4580-4410		cal. 2680 -2400 BC (90.6%)
							cal. 2380-2340 BC (1.9%)

Table 3 Radiocarbon dates from Lanton Quarry

The probability calibration curves are presented in Figure 6 showing 68.2% and 95.4% probability. Only the 95.4% probability age ranges were used for the interpretation of Lanton Quarry.

Figure 6 Probability curves of Lanton Quarry radiocarbon dates.



#### 4.0. Interpretation of Lanton Quarry.

#### 4.1. Radiocarbon Chronology.

The radiocarbon dates from the Lanton Quarry sediment core have produced a chronology that begins in the Late Neolithic 2680 - 2400 cal. BC and extends into the Late Iron Age 370 - 90 cal. BC. Three of the dates display a realistic sequential age depth relationship, whilst one, LAN06 2A (3) appears to be out of sequence. However, this erroneous date most likely reflects intrusive material derived from an episode or phase of high energy fluvial activity that has eroded older sediment from a location up stream and re-deposited the material on the floodplain. The process of periodic sediment recycling within a fluvial system is not unusual and is actually supported by the observations from the pollen analysis of Lanton Quarry. At each analysed level damaged pollen grains were recorded and displayed characteristics such as increased corrosion and crumpling that are indicative of remobilisation of material in a fluvial environment.

#### 4.2. Pollen analysis.

The range of pollen types from Lanton Quarry was relatively diverse and the preservation condition was predominantly "Good", suggesting many grains had not been excessively damaged during transportation, deposition and in the post-deposition environment. However, pollen grains with enhanced damage were recorded from every level analysed, and the percentage values for each level and category of preservation is displayed in Table 4. The damaged pollen grains most likely reflect remobilisation of upstream sediment due to fluvial activity that is subsequently re-deposited on the floodplain close to Lanton Quarry.

Depth	Good	Crumpled	Corroded	Ruptured	Indeterminable
(mm)	%	%	%	%	%
0.06	43.1	29	15.7	7.8	4.3
130	36.3	25.3	23.2	12.8	2.4
200	57	22.5	11.1	7.3	2.1
340	42.4	26.7	19.8	8.4	2.7
410	29.0	42.3	13.4	12.9	2.5
470	39.9	27.6	18.6	10.8	3.1
540	36.7	29.3	15.5	16.4	2.1
610	50.6	20.8	17.2	9.3	2.2

Table 4. Preservation percentage values for Lanton Quarry.

The range of arboreal types was moderately diverse, and included *Alnus glutinosa* (alder), *Betula* (birch), *Ulmus* (elm), *Salix* (willow), *Fraxinus* (ash), *Pinus* (pine), *Quercus* (oak), *Carpinus betula* (horn beam) and *Tilia* (lime). *Alnus glutinosa* was the most frequently recorded arboreal type, recording almost 60%TLP at points in the pollen diagram. However, the dominance of alder is not unusual in a floodplain context, as it most likely represents damp or wet ground, or riparian woodland, within the sample area. In the early part of the pollen diagram *Tilia* recorded a number of declines. The last significant reduction (*c.* 470mm) was possibly in response to changes in the local hydrology allowing alder to expand, as it is specie that can tolerate and thrive on the wetter areas of the floodplain. *Quercus* (oak) and *Corylus avellana*-type (hazel) also record declines around the 470mm depth, which may be further evidence to support a change to wetter conditions

on the floodplain, as oak can tolerate damp conditions but not waterlogged ground. From  $\iota$ . 340mm depth alder records its maximum value (57%TLP) for the whole pollen diagram, and from this point the arboreal composition does not undergo any significant changes until 130mm depth where a decline in alder from 57%TLP to 30%TLP occurs.

The arboreal pollen describes an area dominated by riparian woodland, but with frequent occurrences of mixed deciduous woodland in close proximity to the core site. *Filicales* and *Polypodium* provide additional evidence for a shaded woodland presence within the sample. These ferns frequently form part of the under-story component to the woodland. Birch values were less than 5% TLP throughout the pollen diagram. However, the birch population would be more likely to be living on the dryer and quick draining slopes of the surrounding upland areas (Mitchell, 1990) and not the modern floodplain.

The herbaceous pollens are the most widely represented types throughout the whole pollen diagram. Poaceae (grasses) are most dominant, followed by Cyperaceae (sedges), and indicate open areas throughout the floodplain environment. Minor increases and decreases are recorded for the grasses and sedges throughout the pollen diagram, which are most likely in response to the changes in alder and hazel content around the core site. The grasses begin to consistently increase from about 300 mm depth, and, along with the sedges, exhibit a significant increase at 130mm depth. This most likely represents the expansion of open ground communities into the space made available by the reduction in alder and hazel, producing the present floodplain vegetation composition. Other herbaceous pollen such as *Artemisia* (daisy family), Rosaceae (including raspberry and blackberries) and *Filipendula* (meadow sweet) are recorded periodically throughout the pollen diagram which adds further weight to the interpretation of wet pasture/damp ground rough ground environments close to the sample site.

Aquatic types such as *Myriophyllum verticillatum* (whorled water-milfoil) and Haloragaceae (water milfoil family) are present periodically throughout the pollen diagram. These types indicate slow moving or still, open bodies of water. An additional indicator of standing water was the occurrence of *Typha latifolia* (reedmace – more commonly known as "bulrush"), which is indicative of fens and swampy environments. The final disappearance of these types around 200mm depth has been interpreted as a change in local hydrological conditions from open bodies of water to wet ground marshy floodplain environments. The open bodies of water may range from a ditch to any larger form of pooled water.

The range of human associated pollen types was not particularly varied throughout the pollen diagram. However, the pollen types recorded do suggest continuous human activity throughout the time period represented by the core sediment. The consistent presence of human indicators such as *Plantago lanceolata* (ribwort plantain), Lactuceae (dandelions) and Ranunculaceae (buttercup family) indicates open landscapes and disturbed ground suggesting a Late Neolithic time frame. This age range is corroborated by the radiocarbon chronology. All pollen samples were taken from sediments that post date the Late Neolithic period of 2680 – 2400 cal. BC (LAN06 2B (1)). The human indicators from Lanton Quarry are not associated with any notable phase of woodland clearance and, therefore, further support the interpretation that people were utilising existing open areas postdating the major Late Neolithic phases of woodland clearance (Tipping 1992; 1996). Pastoral agricultural activity is represented by the occurrence of *Rumex acetosal acetosella* (sheep sorrel and common sorrel) (Behre, 1981) whilst arable practises are suggested by the presence of *Avena/Triticum*-type (oat and wheat).

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The generally good condition of the cereal pollen indicates short transport distances and rapid deposition in anoxic conditions, and indicates arable cultivation close to the core site. Avena/Triticum-type (oat and wheat) pollen was identified at 47 cm depth and one Alnus roundwood macrofossil was removed from same sediment used for the pollen analysis and returned a late Bronze Age date of 1130 – 900 cal. BC. Therefore arable cultivation was occurring during the Late Bronze Age close to the Lanton Quarry site. One pollen grain of Linum catharticum (fairy flax) was recorded from 200mm depth and this may be another human indicator, as it was often used for dying fabrics. However, this type was single grain identification, and this therefore is a tentative interpretation. Fairy flax is also native to sandy substrates and may be a natural component of the floodplain vegetation composition. Based on the pollen assemblage surrounding this level a Late Bronze Age date is suggested. An attempt to date material 1 cm below this level was unsuccessful as the date returned was Early Bronze Age 2210 – 1940 cal. BC. However as described previously, this date most likely reflects an episode of sediment recycling and does not alter the interpretation of consistent human activity through the Late Bronze Age at the Lanton Quarry site.

The non-pollen-palynomorphs recorded throughout the pollen diagram are relatively well represented and support the interpretation produced from the pollen identifications. *Filicales* and *Polypodium* (ferns) are both common components of woodlands and were recorded frequently. The presence of NPP types 36, 114 and 190 reinforce the suggestion of damp to wet/waterlogged ground conditions. NPP type 555 (pupal case of *Trichoptera* – caddisfly) indicate pools of slow-flowing or standing bodies of open water at the site (Van Geel, 1998). Microscopic charcoal concentrations (both <50µm and >50µm) decline through the initial part of the zone, and indicate a reduction in fire activity. From 470mm depth microscopic charcoal concentrations increase throughout all zones of the pollen diagram. However, the increase in charcoal is most likely from a natural cause as there is no associated increase in human indicators.

#### 4.3. Comparison with other pollen diagrams in the region.

The generally good condition and moderate diversity of pollen identified in the analysis indicates this is a complex site and for this reason the cores are particularly interesting and valuable. However, the lack of high-resolution sampling makes it difficult to interpret, as the levels counted for this analysis are not from close sampling (every 5mm). Therefore they represent a lower resolution "skeletal" vegetation history of the area around Lanton Quarry. However, the percentage of trees (1 - 57%TLP) and the repeated presence of disturbance indicators and cereal types suggest partially cleared woodland probably used for grazing and limited arable cultivation. The pollen data indicates a post Late Neolithic date, and this is corroborated by the basal date of 2680 - 2400 cal. BC from the Lanton Quarry core and based on comparison with other dated sequences from the area.

The recorded pollen from this analysis has been compared to Akeld Steads, Swindon Hill, Yetholm Loch and Board Moss (see Fig 6). The sampling resolution employed at these sites, by current standards, was coarse and produced a generalised local description of the pollen spectra; however the pollen data are comparable. The pollen data from these sites records types such as *Alnus, Betula, Corylus avellana*-type, Poaceae, Cyperaceae, *Plantago lanceolata*, Rumex acetosa/ acetosella and Polypodium signifying the pollen recorded in this analysis are consistent with the findings of Borek (1975), Davis and Turner (1978) and Tipping (1994; 1998).

Figure 7. Location of pollen analysis sites.



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The closest pollen diagram is from Akeld Steads (Borek, 1975 of Tipping, 1998) less than 1km to the east of the Lanton Quarry core site. The earliest interpretation of the Akeld Steads pollen at first describes a full Holocene sequence. However, the initial interpretation was not constrained by an extensive dating chronology and, due to this the pollen sequence was re-interpreted by Tipping (1998). The pollen types recorded at Akeld Steads are dominated by Alnus glutinosa but also include, Tilia, Betula and Corylus avellana-type. The Akeld Steads pollen indicates an alder dominated landscape, with prevalent open grass and sedge areas, and fringing mixed deciduous woodland in relatively close proximity. The range of herbaceous pollen types recorded at Akeld Steads is appreciably less abundant that the range recorded in the current analysis, but this is probably largely due to the lower pollen sum used in the construction of the pollen diagram. The percentage TLP for *Alnus* recorded at both Lanton Quarry and Akeld Steads is c. 60%, and demonstrates the local dominance of alder. However, there are notable differences for pollen types such as Cyperaceae, Calluna vulgaris and Tilia. At Lanton Quarry, Calluna vulgaris is only recorded twice as a trace presence compared to the continuous presence recorded at Akeld Steads, whilst Cyperaceae at Akeld Steads maintains a higher occurrence than at Lanton Quarry. However these differences reflect the immediate depositional environment rather than any significant local assemblage variations, as Lanton Quarry is situated on the modern floodplain and Akeld Steads is located within a basin edge peat deposit. Tilia records a maximum of 15% TLP at Lanton Quarry and declines to a trace presence through the remainder of the pollen diagram. This trend is not observed at Akeld Steads, and may reflect a localised difference in vegetation composition between a damp floodplain environment and an acid peat environment. Both Swindon Hill and Yetholm Loch display similar vegetation compositions, with Alnus dominating, but the notable differences are the higher

representations of arboreal types such as birch and oak. The differences in tree types most likely represent the change from lowland to upland environment, where the quicker drying slopes are more favourable for birch and oak.

The human indicator types such as *Plantago lanceolata*, *Rumex acetosa/ acetosella* and cereal types are frequently recorded throughout the Milfield basin, indicating almost constant human activity. The range of human indicator pollen types such as Ranunculaceae and *Rumex acetosa/ acetosella* suggests exploitation of the open landscape for pastoral agriculture. The identification of cereal types at Lanton Quarry and associated radiocarbon date can be used to place the pollen diagram into a chronology. Cereal types were comparatively common from *c*. 2600 cal BC (Tipping 1992) at Swindon Hill. Therefore it is tentatively suggested that arable activity around the Lanton Quarry area may have begun in the late Neolithic *c*.2500 BC, but a confident suggestion for cereal cultivation around the Lanton Quarry site dates to the Late Bronze Age 1130 - 900 cal. BC.

The pollen evidence from this analysis indicates that an alder-dominated landscape was widespread along the floodplain. Mixed deciduous woodland was present but most likely to be in the form of small woodland stands. Open areas of grassland were present and this environment was susceptible to changes in the river channel, and may have been subjected to wet and/or waterlogged conditions, as indicated by pollen such as *Alnus glutinosa*, *Rumex acetosa/ acetosella*, *Filipendula* and *Artemisia*. Human activity was present in the form of disturbed ground with associated pastoral and arable indicators, however, some natural disturbance of the landscape could account for their presence. On balance it has been shown that the Lanton Quarry sequence dates from the Late Neolithic to post Late Iron Age.

#### 5.0. Conclusions

The sequence of vegetation change recorded in the Lanton Quarry core is very interesting and complex. If it were possible to conduct high-resolution analysis (every 5 mm from a contiguous stratigraphic sequence) this would provide a more detailed insight to the environmental and vegetation history of the area. In combination with the high resolution pollen analysis additional radiocarbon dates from identified short lived specie macrofossils would construct a reliable and robust chronology. The Lanton Quarry core has already highlighted and radiocarbon dated human activity and suggests arable cultivation took place around the Lanton Quarry core site. Further examination of the core has the potential to reveal when the riverside environment was first opened and exploited by human activity, as well as documenting land-use change through time. There is pollen in acceptable quantities and suitably well preserved to undertake a detailed highresolution pollen count of the Lanton Quarry. Here, within the counted levels, pollen concentrations varied and this is thought to reflect periods of time where the preservation potential was reduced, most likely by conditions where sediments were exposed to highly oxygenated conditions. There is some evidence for episodes of sediment remobilisation and recycling of older pollen, however, this is clearly recognised and can be accounted for in any future detailed pollen analysis.

#### Appendix A

Summery list of botanical and common names.

Botanical Name	Common English name
Arboreal	Trees
Alnus glutinosa	alder
Betula	birch
Quercus	oak
Pinus	pine
Salix	willow
Fraxinus	ash
Tilia	lime
Carpinus betula	hornbeam
Ulmus	elm
Shrubs	Shrubs
Corylus avellana	hazel
Calluna vulgaris	heather
Ericaceae	heath
Hedera helix	ivy
Lonicera	honeysuckle
Euomymus europaeus	European spindle
Herbs	Herbs
Ambrosia-type	ambrosia family
Anthemis	chamomile
Apiaceae	parsley family
Asteraceae	daisy or sunflower family
Caltha palustris	kingcup or marsh marigold
Cannibis	cannibis
Caryophyllaceae	carnation family
Chenopodiaceae	goosefoot family
Cirsium	thistles
Lactuceae	dandelion family
Taraxacum	dandelion
Brassicaceae	caper family
Filipendula	meadowsweet
Galium	bedstraw, goosegrass, cleavers and
	woodruff family

Lamiaceae	mint family
Poaceae	grasses
Cyperaceae	sedges
Plantago major/minor	greater plantain/
Polygonaceae	buckwheat family
Polygonum aviculare	knotweed
Potentillia	cinquefoil, barren strwberry
Rosaceae	rose family
Scabiosa	teasel family
Serratula-type	daisy family
Urtica	nettle family
Urtica urens	burning nettel
<i>Vicia</i> -type	flowering plant (+140 types)
Artemisia	daisy family
Rubus	bramble family
Plantago coronopus	buck's horn-plantain
Rhinanthus-type	rattle
Centaurea scabiosa	Greater knapweed
Aquatics	Water types
Typha latifolia	reedmace - bulrush
*	
Myriophyllum verticillatum	whorled leaf water milfoil
Myriophyllum verticillatum Haloragaceae	whorled leaf water milfoil water milfoil family
Myriophyllum verticillatum Haloragaceae Human indicators	whorled leaf water milfoil water milfoil family
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type	whorled leaf water milfoil         water milfoil family         oat/wheat
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type Linum catharticum	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type Linum catharticum Plantago lanceolata	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type Linum catharticum Plantago lanceolata Rumex acetosa/ acetosella	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type Linum catharticum Plantago lanceolata Rumex acetosa/ acetosella Ranunculaceae	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family
Myriophyllum verticillatum Haloragaceae Human indicators Avena/Triticum-type Linum catharticum Plantago lanceolata Rumex acetosa/ acetosella Ranunculaceae Non-Pollen-	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum         Athyrium filix-femina	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss         common lady fern
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum         Athyrium filix-femina         Osmunda regalis	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss         common lady fern         royal fern
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum         Athyrium filix-femina         Osmunda regalis         Pteridium	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss         common lady fern         royal fern         bracken
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum         Athyrium filix-femina         Osmunda regalis         Pteridium         Pteropsida monolete	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss         common lady fern         royal fern         farn         frens
Myriophyllum verticillatum         Haloragaceae         Human indicators         Avena/Triticum-type         Linum catharticum         Plantago lanceolata         Rumex acetosa/ acetosella         Ranunculaceae         Non-Pollen-         Palynomorphs         Filicales         Sphagnum         Athyrium filix-femina         Osmunda regalis         Pteriofium         Pteropsida monolete         Polypodium	whorled leaf water milfoil         water milfoil family         oat/wheat         fairy flax         ribwort plantain         common sorrel, sheeps sorrel         buttercup family         ferns         peat moss         common lady fern         royal fern         bracken         ferns

#### Appendix B

#### **Pollen Preparation**

#### **Initial Preparation**

- 1. To each boiling tube weigh out 2gm of sample (weight may vary according to expected pollen)
- 2. Add a small amount of IMS (to stop frothing) and 1ml of conc HCl wait for effervescence to subside. Can add more IMS if necessary. Repeat until no more effervescence on adding the acid. Give it a good shake.
- 3. Centrifuge at 2500 rpm for 10 minutes discard the excess acid. Wash with distilled water to near top of tube and centrifuge at 2500 rpm for 10 minutes discard the supernatant
- 4. Boil the centrifuge tubes in a water-bath at 100  $^{\circ}$ C for 20 minutes, stirring regularly.

#### Screening

- Onto an appropriately numbered funnel a 10µm sieve (nylon cloth) is placed. Check for holes in the nylon cloth. Muslin must be wet. Above this is a 'cut away ' funnel and then a 106 µm brass sieve
- 2. Pour the contents of the beaker through this, collecting the liquor as waste in a bottle. Wash the 106  $\mu$ m sieve with distilled water, collecting the <106  $\mu$ m fraction on the 10 $\mu$ m sieve.
- 3. When the 106- $\mu$ m sieve has been thoroughly washed, then stir the liquor on the 10 $\mu$ m sieve and wash this with distilled water until the waste is clear.
- 4. Wash the residue towards the lip of the sieve, and then using a wash bottle fitted with a jet, wash this entire residue into the 10 ml centrifuge tube.
- 5. Centrifuge the tubes at 2500 rpm for 10 minutes. Discard the supernatant.
- 6. Retain the  $10 \,\mu\text{m}$  sieve as this is used again after the hydrofluoric acid stage

The macro on the 106  $\mu m$  sieve is washed into a 100 ml bottle and retained for possible analysis

Use the sieve upside down over the sink.

The brass sieves are then placed in an ultrasonic bath for thorough cleaning before being soaked overnight in peroxide.

#### Acetolysis

## The acetolysis mixture reacts VIOLENTLY with water and so great care must be exercised, again using a fume cupboard and full personal protection.

The Acetolysis Mixture is 9 parts Acetic Anhydride + 1 part conc. Sulphuric Acid This should be prepared by a Technician using a fume cupboard

- 1. About 2 ml of Glacial Acetic Acid (red wash bottle) is added to the residue. The tube is then centrifuged at 2500 rpm for 10 minutes and the supernatant is discarded into a tub of water.
- 2. Stage 1 is then repeated to ensure that all water is removed
- 3. About 6 8 ml (half way up test tube) of the Acetolysis Mixture is carefully added to the residue
- 4. After stirring the tubes are heated at  $100 \, {}^{0}$ C for 5 10 minutes in a waterbath. This is the absolute maximum or the sample will char.
- 5. The tubes are then centrifuged at 2500 rpm for 10 minutes
- 6. The supernatant is then cautiously discarded into a <u>DRY</u> beaker and disposed of by the Technician.
- 7. If the organic content of the sample is high then repeat stages 3 6
- 8. Ca. 2 ml of glacial acetic acid (red wash bottle) are added to the residue. The tubes are centrifuged at 2500 rpm for 10 minutes and the supernatant discarded.
- 9. The residue is now washed with distilled water, (near to top of test tube) centrifuged and then the supernatant discarded.
- 10. Stage 9 is then repeated.
- 11. Dispose of excess acetolysis mixture into sink with water running.

#### Additional density separation stage

- 1 Personal protective clothing was worn throughout the preparation; lab coat and gloves at all times and goggles when handling chemicals. All procedures involving chemicals were carried out in the fume cupboard and the various supernatants were decanted down the sink in the fume cupboard, using running water, both before and after disposal.
- 2 All laboratory equipment was washed with distilled water between samples, and capped centrifuge tubes were used following the sieving stage. Where necessary, any residues or supernatants were transferred and washed using distilled water, and unless otherwise stated centrifugation was carried out for five minutes at 3000 rpm. Residue pellets were suspended using the vortex, or in the most persistent samples they were mixed with a disposable wooden stick. The following treatments were carried out in sequence:

#### Sampling and addition of a known quantity of exotic spores

The soils were sampled using a plastic syringe to take 1ml of soil. Each sample was then placed in a marked test tube with a single *Lycopodium* tablet (Batch no. 307862 where the average number of spores per tablet is 13 500). 1ml each of distilled water and 10% hydrochloric acid (HCl) was added to dissolve the tablet.

#### Disaggregation and sieving of the sediment

When the effervescence had stopped, 6ml of 10% sodium hydroxide (NaOH) was added and the test tubes were placed in a hot water bath for 15 minutes and stirred occasionally with a glass rod. The disaggregated samples were then sieved using 10µm mesh and 125µm sieve, tripod, funnels and distilled water, and the residue transferred to a marked centrifuge tube, centrifuged and decanted. The residue was then washed with distilled water.

#### Heavy liquid separation to remove the heavier mineral fraction

5 5ml of sodium polytungstate  $(Na_6(H_2W_{12}O_{40}).H_2O)(1.92 \text{ mg/ml})$  was added to each tube. The residues were then suspended using the vortex for 1 minute per sample, and centrifuged for 20 minutes. The supernatants containing the organic fraction were then decanted into fresh labelled tubes. These tubes were topped up with distilled water and the organic residue suspended using the vortex for 1 minute, before being centrifuged for 10 minutes. The residues were decanted and washed with distilled water.

#### Acetolysis to remove cellulose

6

10ml of glacial acetic acid (CH<sub>3</sub>COOH) was added to the residues in each tube which were suspended and then centrifuged and decanted.

[The acetylation mixture was made up fresh by mixing acetic anhydride  $((CH_3CO)_2O)$  and concentrated sulphuric acid  $(H_2SO_4)$  in proportions 9:1 by volume. The required volume of acetic anhydride  $((CH_3CO)_2O)$  was measured first, then the corresponding volume of concentrated sulphuric acid  $(H_2SO_4)$  was added (dropwise). This was done very carefully, stirring continuously to prevent heat building-up, and stirred again before use].

7ml of the acetylation mixture was added to each sample residue and stirred gently with a glass rod. The rods were then removed and the open tubes were then placed in a boiling water bath for 1-2 minutes (stirring is unnecessary at this stage and the glass rods were removed from the tubes to prevent steam from condensing and running down into the mixture and reacting violently). One minute is usually adequate, as longer acetylation can make the pollen grains opaque. The tubes were removed from the water bath and topped up with glacial acetic acid in order to stop the acetylation process. The screw caps were then replaced and the tubes gently inverted to mix the contents before being centrifuged and decanted. The supernatants from this stage were decanted into a large (1000ml) beaker of water before being poured down the sink in the fume cupboard. 10ml of glacial acetic acid (CH<sub>3</sub>COOH) was then added to each residue, which was suspended, centrifuged and decanted. Each residue was then washed twice in distilled water.

#### Dehydration and suspension in silicone oil

7 5ml of industrial methylated spirits was added to each residue, which was suspended, centrifuged and decanted. Then 2ml of tertiary butyl alcohol ( $C_4H_9OH$ ) (TBA) was added to each tube. The samples were suspended, centrifuged and decanted. The residues were transferred to labelled vials using individual glass pipettes, and then an equal volume of silicone oil was added using a glass rod. The contents were mixed using wooden cocktail sticks and the vials were left in a warm oven (55°C) for 36 hours to promote the evaporation of the TBA.

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