Fossil oak galls preserve ancient multitrophic interactions

Graham N. Stone1,* , Raymond W. J. M. van der Ham2 and Jan G. Brewer3

1Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK
2Nationaal Herbarium Nederland, Universiteit Leiden, PO Box 9514, 2300 RA Leiden, The Netherlands
3Hogebroeksweg 32, 8102 RK Raalte, The Netherlands

Trace fossils of insect feeding have contributed substantially to our understanding of the evolution of insect–plant interactions. The most complex phenotypes of herbivory are galls, whose diagnostic morphologies often allow the identification of the gall inducer. Although fossil insect-induced galls over 300 Myr old are known, most are two-dimensional impressions lacking adequate morphological detail either for the precise identification of the causer or for detection of the communities of specialist parasitoids and inquilines inhabiting modern plant galls. Here, we describe the first evidence for such multitrophic associations in Pleistocene fossil galls from the Eemian interglacial (130 000–115 000 years ago) of The Netherlands. The exceptionally well-preserved fossils can be attributed to extant species of Andricus gallwasps (Hymenoptera: Cynipidae) gallowasting Quercus, and provide the first fossil evidence of gall attack by herbivorous inquiline gallwasps. Furthermore, phylogenetic placement of one fossil in a lineage showing obligate host plant alternation implies the presence of a second oak species, Quercus cerris, currently unknown from Eemian fossils in northwestern Europe. This contrasts with the southern European native range of Q. cerris in the current interglacial and suggests that gallwasp invasions following human planting of Q. cerris in northern Europe may represent a return to preglacial distribution limits.

Keywords: cynipid; fossil; Pleistocene; multitrophic; gall; oak

1. INTRODUCTION
Trace fossils made by feeding insects have played a major role in our understanding of the evolution of insect–plant interactions (Labandeira & Phillips 1996; Wilf et al. 2001, 2006; Currano et al. 2008). The most structurally complex signs of insect activity in plant tissues are galls, plant tissues whose development is controlled by the gall-inducing organism (the galler) and which provide the galler with nutrition and protection (Cornell 1983; Price et al. 1987; Stone & Schönrogge 2003). The insect control of gall morphology is such that galls are the extended phenotypes of galler genes (Crespi & Worobey 1998; Stone & Cook 1998), and many galls can be identified to species on the basis of gall morphology alone (Raman et al. 2005). Although three-dimensionally preserved insect-induced galls, dated at ca 305 Myr, are known, most are two-dimensional impressions whose lack of morphological characters precludes more specific identification of the causer (e.g. Scott et al. 1994; Labandeira & Phillips 1996). However, the inducers of well-preserved and structurally complex galls can sometimes be identified with high taxonomic resolution (e.g. Dieguez et al. 1996; Waggoner 1999; Erwin & Schick 2007).

In addition to the causer, many galls support communities of specialist herbivorous inquilines and natural enemies. The herbivorous inquilines are obligate inhabitants of galls induced by specific host galler species, and though they do not feed on the gall, their activities can cause its death either directly or indirectly (Shorthouse 1980; Washburn & Cornell 1981; Askew 1984; Wiebes-Rijks & Shorthouse 1992; Ronquist 1994). The inquilines are often closely related to the gall inducers whose galls they attack (Ronquist 1994; Crespi & Abbot 1999; Miller & Crespi 2003), and this association (termed agastoparasitism by Ronquist 1994), closely parallels true parasitism. Both gall inducers and inquilines are attacked by natural enemies, particularly parasitoid wasps, and all three trophic groups commonly leave characteristic signatures in gall tissues (Stone et al. 2002; Raman et al. 2005). Fossil galls thus have the potential to provide direct evidence of within-gall multispecies associations. Here, we describe the first evidence for such associations, in Pleistocene fossil galls from the Eemian interglacial (130 000–115 000 years ago) whose excellent three-dimensional preservation not only allows precise identification of the causers but also provides the first fossil evidence of gall attack by specialist inquilines. Furthermore, phylogenetic placement of one of the Eemian gall causers in a lineage showing highly conserved host plant associations allows new palaeobotanical inferences for northwestern Europe that provide a striking contrast with the current interglacial.

2. FOSSIL MATERIAL STUDIED
The fossil galls (figures 1 and 2) were discovered in a gravel pit near Raalte, Overijssel Province, The Netherlands (see van der Ham et al. (2008) for a description of the site and associated biological material). They were preserved in Late Eemian (ca 125 000 BP) sediments of the Kreftenheye Formation (de Mulder et al. 2003) laid down by the ancient Rhine in the valley of the present river Ijssel (van der Ham et al. 2008). Eemian vegetation at the site comprised temperate woodland including wetland...
trees (e.g. Alnus, Populus and Salix) and upland forest species (e.g. Abies alba, Acer, Carpinus betulus, Ilex aquifolium and Quercus). The fossils are of two types.

(i) **Type 1.** The most abundant type ($n=47$; figure 1) is preserved as a slightly (figure 1b) to moderately (figure 1c) compressed structure, originally approximately spherical and 37–57 mm in diameter. The original outer gall surface is exceptionally well preserved and marked with small regularly spaced tubercles or parallel ridges (figure 1a). The fossils have a hollow interior (figure 1b,c) and, when complete, contain a single thin-walled chamber 5–6 mm long (figure 1d) that in some cases is attached to the interior wall on each side of its long axis (figure 1c). In all cases, this chamber has a smooth-edged hole 2 mm in diameter at one end. Three fossils have holes in the exterior (arrowed in figure 1e,f); each has a single larger slightly lenticular hole (figure 1f; dimensions $1.9 \times 0.8$ mm, $3 \times 1.2$ mm and $1.9 \times 1.0$ mm), while two galls have 1–3 smaller circular holes 0.6–0.8 mm in diameter (figure 1e,f).

Figure 1. Type 1 gall fossils. (a) External views (scale in cm). (b) Longitudinal section showing internal airspace, with inner larval chamber missing. The gall’s point of attachment is to the left. (c) Two halves of a sectioned compressed gall, showing the larval chamber in the left of the section. (d) A larval chamber, with the adult emergence hole to the right and (inset) in end-on view (scale in mm). (e,f) Two views of the same fossil gall, showing multiple emergence holes (arrowed). In (e), two of the small emergence holes are shown in enlarged view (boxed).
Type 2. A single example (figure 2a, b) was found of a second type. One surface lacks obvious structure but bears several small holes approximately 1 mm in diameter (arrowed, figure 2a). The opposite surface comprises an aggregation of thin-walled chambers (figure 2b) approximately 5 mm long, joined in one case by a smoothly rounded hole 2 mm in diameter (arrowed, figure 2b).

3. DIAGNOSIS

Both fossil types can be unambiguously identified as galls induced by oak gallwasps (Hymenoptera: Cynipidae) on the basis of striking similarity to modern forms. No other gall inducers produce galls of this size and complexity in the Western Palaearctic (Docters van Leeuwen 1957; Buhr 1964–1965). Oak cynipid galls are diagnostic not only of the gallwasp species but also of alternating sexual and asexual generations in a parthenogenetic life cycle (Stone & Cook 1998; Stone et al. 2002, 2008). Phylogenetic relationships, oak host associations and associated gall communities of the Western Palaearctic gallwasp fauna have been studied in depth (Stone & Cook 1998; Cook et al. 2002; Rokas et al. 2003b), allowing extensive inference of biology and associated communities from gall structures. Studies of within-species genetic diversity show that all modern Western Palaearctic oak gallwasp species so far studied are at least 1–2 Myr old, and so long predate the Eemian (Rokas et al. 2001, 2003a; Stone et al. 2002, 2007; Challis et al. 2007). The Raalte fossils are thus certainly young enough to be attributable in principle to extant species.

Type 2. The clustered cells in this fossil most closely resemble the asexual generation galls of Andricus quercusradicis (figure 2c). These galls comprise multiple aggregated larval chambers within a woody outer layer lacking distinctive surface sculpture, reaching a total diameter of 80 mm (Docters van Leeuwen 1957; Ambrus 1974). We interpret the type 2 fossil as a fragment of such a gall, whose exterior bears the small holes in figure 2a. The quality of preservation of the type 1 galls suggests that remnants at least of any more distinctive surface structures would be visible. Other woody, multichambered Western Palaearctic cynipid galls

**4. DISCUSSION**

**a. Multispecies interactions**

Both gall types show holes in the gall surface and, for the type 2 fossil, between internal larval chambers. These holes are characteristic of those made by emerging adult members of the gall community. The single larger surface holes on type 1 galls match the diameter of the aperture in the single inner larval chamber (figure 1d), and were chewed by an emerging gallwasp. Gallwasps emerging from multichambered galls commonly chew their way through vacated surrounding chambers, explaining the larger internal hole in the type 2 fossil. The smaller surface openings are too small to have been made by the galler in either case. Oak cynipid galls are commonly occupied by inquilines cynipids (Cynipidae: Synergini) that cannot induce their own galls but inhabit those induced by other causers, predominantly gallwasps (Stone et al. 2002). The inquilines are always smaller than the gall inducer and induce their larval chambers either within the host larval chamber (in which case the galler is always killed) or in the outer gall tissues (Washburn & Cornell 1981; Askew 1984; Wiebes-Rijs & Shorthouse 1992; Ronquist 1994; Stone et al. 2002). The presence of both large and small emergence holes in a single type 1 gall can only be attributed to attack by outer gall inquilines. All outer gall inquilines in modern oak cynipid galls are species in the genus *Synergus*, and the small emergence holes must lead to chambers induced by Eemian *Synergus*. *Synergus* inquilines are themselves attacked by chalcid parasitoids (Askew 1961, 1984; Schnörrge et al. 1995, 1996a,b) and the smaller emergence holes could thus have been made either by emerging adult inquilines or parasitoids. Both of these trophic groups are associated with modern *A. hungaricus* galls (Melika et al. 1997). The many-chambered structure of the type 2 fossil makes the identification of causers of the smaller surface emergence holes less certain: though too small to have been made by the galler, they could have been made by emerging inquilines or by parasitoids of either inquilines or gellers.

emergence holes in the Raalte fossils were made by chalcid parasitoids. The precise identification of the gall inducer from the gall phenotype, and hence a prior expectation of the relative sizes of the gall inducer and other gall inhabitants, was central to our interpretation of the trace fossils. It is possible that other fossil galls showing apparent emergence holes that are small relative to the size of the gall may represent evidence of inquiline or parasitoid attack: candidates include galls DT83 and DT84 in the web-based Guide to Insect (and Other) Damage Types on Com-pressed Plant Fossils athttp://paleobiology.si.edu/insects/
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pressed Plant Fossils at http://paleobiology.si.edu/insects/
index.html (Dr C. Labandeira 2008, personal communi-
cation). However, many large galls house multiple small
gall inducers (see Raman et al. (2005) for examples in many
arthropod groups), and the relative size of galls and
emergence holes may be an unreliable guide to the presence
of non-galling inhabitants. Nevertheless, we expect that the
examination of other fossil galls attributable to extant gallers
taxa, whose associated communities are well known, will
yield further evidence of dependent trophic interactions.

(b) Oak gallwasp s as indicators of past flor as

Andricus hungaricus lies within a clade of Andricus species
whose lifecycles require obligate alternation between two
different taxonomic sections of the genus Quercus (Cook
et al. 2002; Stone et al. 2008). The asexual generation
always galls section Quercus sensu stricto oaks (such as
Q. robur and Quercus petraea), while the sexual generation
always galls section Cerris oaks (particularly Turkey oak,
Quercus cerris). The most recent common ancestor of this host-alternating clade long predates the Pleistocene
(Cook et al. 2002), implying that the Eemian causer of
gall type 1 was also a host alternator. This allows a novel
collembanonical inference, because while the presence of
section Quercus oaks at the Raalte site is confirmed by
pollen and macrofossils (van der Ham et al. 2008), there is
direct evidence for section Cerris oaks. The type 1 fossils
imply that during the relatively short (ca 15 000 years)
Eemian interglacial, both oak sections and their associated
insects escaped their southern European glacial refugia
(Petit et al. 2002) to colonize northern Europe. The
identification of the type 2 fossil provides additional
(though weaker) support for this conclusion, for although
both generations of A. quercusradicis can be found on
section Cerris and section Quercus, the sexual generation is
most commonly associated with section Cerris (Ambrus
1974; Melika et al. 2000).

(c) Implications for modern gallwasp distributions

The presence of section Cerris oaks inferred from the Raalte
fossils contrasts with the oak flora native to the same region
in the current interglacial. Only two section Quercus oaks,
Q. robur and Q. petraea, are native to northern Europe,
while section Cerris oaks (and hence associated gallwasp s)
are restricted to southern Europe and predominantly to
regions south of the Pyrenees, Alps and Carpathians
(figure 4). More specifically, A. hungaricus is now restricted
to a region far to the south and east of its Eemian
distribution, in Hungary and the Balkan refugium (figure 4;
Ambrus 1974; Melika et al. 2000). However, over the last
500 years, human planting has extended the distribution of
Q. cerris far beyond its natural distribution, and it is now
naturalized as far north and west as Ireland and Scotland
(Walker et al. 2002). Northwards range expansion by
Q. cerris was thus prevented not by physiological limi-
tations, but by inability to escape its Pleistocene glacial

Figure 4. Location of the Raalte sampling location (white circle) relative to modern natural distributions of the oak sections
| Quercus sensu stricto and Cerris. Line 1 represents the northern limit of oaks in the section Quercus and of all oaks in the Western
| Palearctic. Only section Quercus oaks are native between lines 1 and 2. Line 2 represents the northern limit of the natural distribution of section Cerris oaks. Oaks in sections Quercus and Cerris are naturally found together between lines 2 and 3. Line 3 represents the southern limit of oaks in the section Quercus. Only section Cerris oaks are found between lines 3 and 4. Line 4 represents the southern limit of section Cerris oaks. Distributions are based on regional floras as explained in Stone et al. (2007).
refugia (Svenning & Skov 2004). The introduction of Q. cerris has in turn triggered invasion of northern Europe by multiple host-alternating gallwasp and their natural enemies (Stone et al. 2002; Hayward & Stone 2006), with potential for major direct (competition, parasitoid attack) and indirect (apparent competition mediated by shared enemies) impacts on native communities (Schönhøgge et al. 1995, 1996a,b, 1999, 2000; Stone et al. 1995; Atkinson et al. 2002). At least four host-alternating species are now established in The Netherlands (Andricus corruptrix, Andricus kollari (figure 3, gall 1), Andricus lignicolus (figure 3, gall 2), Andricus quercuscalcius (figure 3, gall 9): Docters van Leeuwen 1957; Stone & Sunnucks 1993; Stone et al. 2007). The Raalte fossils imply that these gallwasp, though anthrotopogenous invaders in the current interglacial, were native to northwestern Europe in the previous interglacial. Their current range expansion should thus perhaps be considered as a return to preglacial distribution limits and ecology.

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