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Ancient death-grip leaf scars reveal ant–fungal parasitism

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Parasites commonly manipulate host behaviour, and among the most dramatic examples are diverse fungi that cause insects to die attached to leaves. This death-grip behaviour functions to place insects in an ideal location for spore dispersal from a dead body following host death. Fossil leaves record many aspects of insect behaviour (feeding, galls, leaf mining) but to date there are no known examples of behavioural manipulation. Here, we document, to our knowledge, the first example of the stereotypical death grip from 48 Ma leaves of Messel, Germany, indicating the antiquity of this behaviour. As well as probably being the first example of behavioural manipulation in the fossil record, these data support a biogeographical parallelism between mid Eocene northern Europe and recent southeast Asia.

Keywords: ant–fungal parasitism; tritrophic interaction; middle Eocene; Messel

1. INTRODUCTION

Many parasites have evolved the ability to manipulate host behaviour in ways that promote parasite fitness [1]. One of the most impressive examples of adaptive manipulation of host behaviour is the death grip of ants infected by the fungus *Ophiocordyceps unilateralis*. In this system, ants attach to major leaf veins along their abaxial surface by their mandibles before being killed by a fungal parasite. This fungus rapidly grows a stalk from the ant's head, producing an ellipsoidal body from which spores are released to infect other ants [2]. In one well-studied population in southern Thailand, the fungus was very specific and 97 per cent of hosts belonged to a single ant species, the carpenter ant *Camponotus leonardi* [3]. The manipulation of ant behaviour is precise, with infected workers leaving their colonies in the high canopy of tropical forests (greater than 20 m) to bite into leaves within a narrow zone 25 cm above the forest floor [4]. Local densities of dead ants attached to leaves can be high, with up to 26 m⁻² [3]. The manipulation of worker ant

behaviour was demonstrated to be adaptive for the fungus as it functions to place and secure an ant in a distinct niche that has optimal humidity and temperature for the growth of the fungus from the post-mortem ant carcass [4]. For the leaf that becomes a platform for this manipulation, the relevant observation is that infected ants almost always (98%) bite into major leaf veins on the abaxial surface.

Unusual insect behaviour and its consequences occasionally are preserved in the fossil record, but previously have not implicated parasites as agents of behavioural modification. Many fungi manipulate insects to bite leaves in a similar manner to *O. unilateralis* [5] and so this type of manipulation is a probable candidate to be captured in the fossil record through the examination of fossil leaves. Here, we report physical evidence for 'death grip' scars on damaged leaf tissue, induced by stereotypical control of ant behaviour by fungal infection. The stereotyped pattern of leaf damage left by the ant death grip is a crucial part of the fungal life cycle and provides evidence for the presence of this spectacular association in subtropical forests surrounding Lake Messel 48 Ma. It also indicates that the fossil record of plant–insect associations can cascade up and down three levels.

2. MATERIAL AND METHODS

(a) Fossil samples

The Messel Pit (Grube Messel) fossil site is located on the eastern side of the Rhine Rift Valley in Hesse, Germany, about 8 km north-east of the town of Darmstadt (49°55' N, 8°45' E). The site is famous for the exceptional preservation of its fossils and was therefore declared an UNESCO Natural Heritage Site in 1995. Currently, it is a large pit with a diameter of about 0.7 × 1.0 km. The former lake basin is supposed to have had a diameter of about 1.5 km and initially was up to 300–400 m in depth [6,7]. The water body of the former lake was meromictic through chemical and thermal stratification. The sedimentary basin fill is known as the Messel formation. It is comprised of mostly laminated claystone sediments ('oilshale') and is more than 140 m thick, representing approximately 1 Myr of deposition [6]. Radiometric dating of basalt fragments underlying the fossiliferous sediments indicates that the deposits have an age of 47.8 ± 0.2 Myr [8]. The oilshale sediments are richly fossiliferous, and have yielded a highly diverse flora and fauna. Insects are among the most frequently found fossils, documenting a very diverse, mostly terrestrial fauna [9]. The examined specimen (SM.B.Me 10167) is housed in the Messel plant collection of the Forschungsinstitut Senckenberg, Germany. Examination was made with a Leica MZ12.5 stereomicroscope.

(b) Modern samples

Workers of the ant *C. leonardi* killed by *O. unilateralis* were collected from the underside of leaves in the Peninsula Khao Chong Botanic Garden, ca 20 km east of Trang in southern Thailand (7°32'49.50" N, 99°47'14.73" E). Collections took place during 2007. A section of the leaf to which the ant was attached was cut off and the leaf and ant–fungus were oven dried at 55°C for 12 h and then placed individually in dry sealed boxes to prevent decomposition.

The ant was gently removed from the leaf using forceps and the leaf tissue damage was assessed using a standard dissecting scope and imaged using a JVC KYF70B digital camera mounted on a Leica MZ12.5 stereomicroscope. Because of the inherent problem of extremely shallow depth of field in microphotography, images are composites between 15 and 35 individual shots taken at different focal points. These images are then combined using the AUTO-MONTAGE software package from Syncroscopy.

3. RESULTS

A unique fossil example of this association, indicating fungal manipulation of an ant host's mouthparts, was found on a single leaf of the dicotyledonous plant host *Byttneriopsis daphnogenes* (Ettinghausen) Kvaček

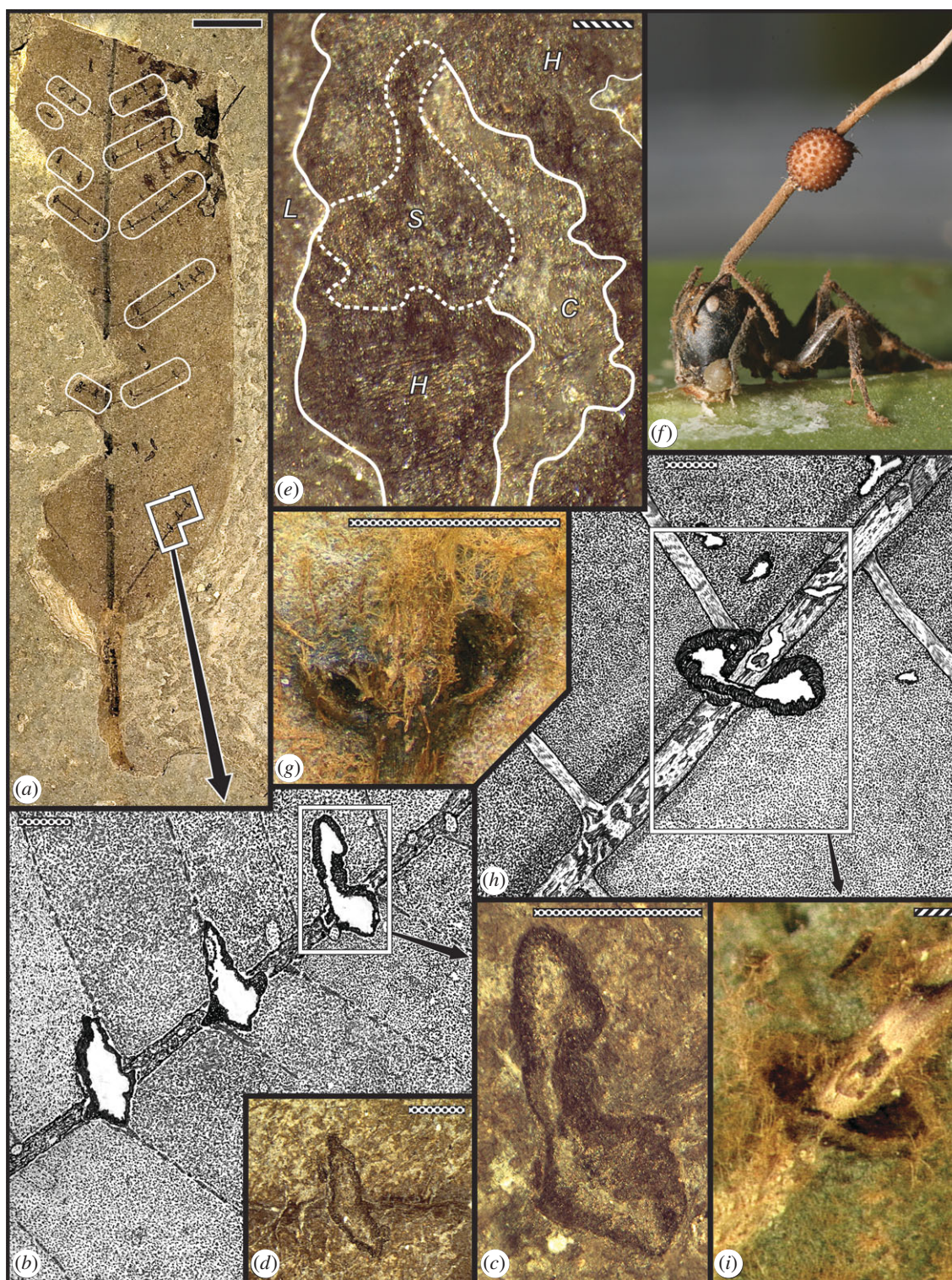


Figure 1. (a) A nearly complete fossil leaf (SM.B.Me 10167) from Messel with 29 ant death-grip scars centred on 11 secondary veins (rectangular templates). (b) Overlay drawing and enlargement of three of four scars of (a) showing shape, callused periphery and relationship of secondary to tertiary venation. (c) Enlargement of upper-right scar in (b), showing dumbbell-shaped hole and central concavity of callused tissue. (d) Additional but narrower scar with less pronounced dumbbell. (e) Detail of a scar from the fossil leaf at (a), showing unaffected leaf tissue (L); callused region showing hyperplastic cell files radiating from central cut (H); texturally distinct fungal infection of callused tissue seen on the surface (S); and central cut area (C). (f) Modern plant specimen [4], showing a mature *O. unilateralis* stroma issuing from the head of a dead *C. leonardi* whose mandibles are attached to the lower surface of a major vein. (g) Modern ant death-grip scar from a primary-secondary vein axil, showing the medial vein, dumbbell-shaped hole, callus rim and hyphae from later fungal colonization. (h) Overlay drawing of a second, modern leaf specimen with a death-grip scar at secondary and tertiary axil; other holes may be aborted mandibular punctures. (i) Photo showing same features in (h). Scale bars: solid bar, 1 cm; stippled bar, 1 mm; slashed bar, 0.1 mm.

et Wilde comb. nov. (Malvaceae; figure 1*a*) from Messel in north-central Germany [10]. It was initially considered an example of vein-cutting behaviour involved in latex draining, a common modern behaviour resulting in damage from herbivorous insects [11]. This hypothesis was discounted because the leaf bore different, dumbbell-shaped mandible cut marks and lacked the distinctive snips that would result from vein-cutting behaviour. Also, the host-plant taxa are latex-bearing lineages.

The close modern parallel for this distinctive type of leaf damage is the death grip of some fungus-infected carpenter ants. In the best-documented interaction (figure 1*e*), the fungus *O. unilateralis* adaptively manipulates worker ants of *C. leonardi* to bite along major veins of leaves in Thai tropical forests [4]. The effect on the leaf of this death-grip behaviour is a pair of widened mandibular puncture marks surrounded by callused tissue that occur on each side of a primary or secondary vein (figure 1*f–h*). Importantly, bites left by the ant on the leaf blade are distinctive and centred on major veins; the choice of leaf-host taxon by the ant is non-selective and based overwhelmingly on environmental cues of favourable temperature and humidity for growth of its fungal parasitoid near the forest floor [4].

Manipulation of worker ant behaviour by the fungus *O. unilateralis* is host specific and occurs only on three genera in the tribe Camponotini [12]. Most hosts occur in the hyperdiverse genus *Camponotus*. Although *Camponotus* fossils are not known from Messel, this genus is found in Eocene Baltic amber, also from Germany, and in coeval Rovno amber from Ukraine [13], and a geochronologically more close (Middle Eocene) deposit (albeit in the USA, [14]) suggesting that this genus may also have occurred in the Messel palaeoecosystem.

4. DISCUSSION

Evidence for insect–fungal–plant associations heretofore have been absent from fossil compressions, principally because such an association would rely on detection not from the separate insect and fungus fossils, but rather preservation of a distinctive association evidenced by insect damage on fossil leaves. Another, albeit undocumented, example is a galled Patagonian leaf taxon with insect galls that contain several parasitoid emergence holes [15].

Generally, amber dominates the fossil record of pathogenic fungi on insect hosts [16]. The oldest occurrence consists of two conspicuous stalks of the ascomycete *Paleoophiocordyceps coccophagus* projecting from the head of a male scale insect in Early Cretaceous Burmese amber [17] at about 105 Ma. In more recent, early Miocene Dominican amber, approximately 21 Ma, conidiophores, attributable to the zygomycete *Entomophthora*, enveloped a termite carcass [18]. However, in both cases no evidence of behavioural modification can be seen.

The dating of this unique parasitic association minimally to the mid Palaeogene, indicates a deep-time origin for this phenomenon. The modification of a fungally-induced behaviour in this ant taxon now has an origin occurring after ant lineages had diversified

and at a time when they were becoming numerically dominant [19]. Consequently, this highly specialized interaction is relatively ancient and not a recent acquisition.

Evidence from middle Eocene sites in Europe, especially from Baltic Amber indicates considerable sharing of plant and insect taxa with modern southeast Asia [20]. The current study adds to that growing list, suggesting that the forests of Thailand may be a close analogue to Lake Messel of the mid Eocene (47.8 Ma). Our results offer unique information in the newfound use of distinctive, stereotypical damage that mirrors associations that are documented, often meticulously, from modern material.

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