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Animal-mediated fertilization in bryophytes – parallel or precursor to insect pollination in angiosperms?

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Bryophytes have until now been absent from all hypotheses regarding the origin of insect pollination of seed plants. The discovery that both springtails and mites are able to transfer sperm in mosses makes it possible to suggest a 'Bryophyte precursor hypothesis of plant pollination' stating that animal-mediated fertilization in mosses and insect pollination in seed plants is historically linked by homologous or analogous evolution of structures responsible for attraction and reward aimed at a pool of fertilization vectors originally co-evolved with bryophytes and subsequently co-opted by seed plants. The earliest point in time when animal-mediated fertilization of bryophytes, involving springtails and mites, could have arisen is Late Silurian/Early Devonian, ca 280 Myr before the evolutionary radiation of angiosperms.

Soil-dwelling microarthropods and wingless insects are assumed to have been the primary vectors transporting sperm in ancient mosses and possibly other groups of bryophytes. These animals may later have secondarily expanded to transfer pollen in gymnosperms and angiosperms, similar to the frequent pollinator shifts observed among angiosperm groups. Several ancestral traits pointed out by recent studies of early angiosperms are in favour of this hypothesis, such as small size of flowers, small and non-sticky pollen, adaptations for growth in moist and shaded habitats close to running water, and weedy growth forms with rapid life-cycles in disturbed habitats. It is also possible to envisage a link from sexual attractants excreted by oogonia in charophyte algae and archegonia in bryophytes to guide sperm, via sugar released to attract insects in pollination drop mechanisms in gymnosperms to more advanced structures specialized for insect attraction such as nectaries and nectar discs in angiosperms.

In a paper published in Science (Cronberg et al. 2006), we were able to demonstrate that fertilization of the moss *Bryum argenteum* can be mediated by microarthropods (springtails and mites). In a series of preference tests we also showed that these animals tend to visit fertile rather than sterile shoots. Recently, Rosenstiel et al. (2012) in a paper published in Nature have shown that fertilization is improved in mosses by springtails even when water is present. They also identified a large number of volatile substances that are emitted from fertile shoots and demonstrated that the springtails are guided by scent to the fertile shoots. Taken together these results point towards a fertilization syndrome that is comparable to insect pollination in angiosperms.

Mosses and other bryophytes (liverworts and hornworts) are much older groups of plants than angiosperms,

and the two groups of microarthropods (springtails and mites) also belong to groups with an ancient evolutionary history, much older than modern pollinating insects. One of the major implications of our study was that 'pollination-like' plant–animal associations occurred long before the advent of angiosperms. This point was perhaps one of the major reasons that motivated Science to publish our study and it was subsequently picked up by the secondary press. For example, the American popular science magazine 'Discovery Magazine' in their annual review of major scientific 'stories' for 2006 listed our study as number 46 in Science and number 4 in Biology, because it potentially pushed the origin of pollination biology many million years back in time.

The algal ancestors to all land plants are found among the charophyte algae, with stoneworts (*Chara*, *Nitella* etc.)

and the genus *Coleochaete* as the present-day sister groups to all land plants. These algae are fertilized by sperm, which can freely swim between male and female structures in the aquatic environment. All early-divergent land plants, such as ferns, lycopods, horsetails and bryophytes (mosses, liverworts and hornworts) are still fertilized by sperm, whereas fertilization in gymnosperms and angiosperms is mediated by pollen, which is drought resistant and dispersed by wind or animals.

The life cycle of bryophytes, revealed by Wilhelm Hofmeister (Hofmeister 1851), thus involves fertilization by motile sperm, of size 20–30 μm (Renzaglia et al. 1995). The fertilized egg cell develops into a sporophyte, which is physically attached to the female gametophyte. Observations of sperm suggest a speed of up to 100–200 $\mu\text{m s}^{-1}$ (Richards 1978), but their movement is erratic (Muggoch and Walton 1942) and water surfaces are often discontinuous. Consequently, fertilization distances are normally restricted to few centimetres (Longton and Schuster 1983). The dispersal range can be further increased by physical factors like flowing water or the energy of falling raindrops when hitting discoid perigonia, so called 'splash-cup' structures, cf. Longton and Schuster (1983) and Andersson (2002). Estimates of fertilization distances have usually been based on field observations of the physical distance between a female with sporophyte and the closest located male (Longton and Schuster 1983), and do not reveal the degree of animal contribution to sperm transfer.

Traditionally, the origin of animal-mediated fertilization has been assumed to involve angiosperms and insects (insect pollination). However, angiosperms first emerged during the early Cretaceous (ca 140 million years ago) and some of the insect groups involved in pollination appear to have radiated well before this period (Willis and McElwain 2002, Labandeira et al. 2007). Gymnosperms have historically been considered to be exclusively wind-pollinated, but later studies have demonstrated that insect-pollinated representatives occur in at least two orders, Cycadales (Norstog et al. 1986) and Gnetales (Bino et al. 1984, Kato and Inoue 1994). It has been hypothesized that insect pollination started as pollinivory (pollen consumption) and then evolved towards more complex mutual relationships (Labandeira 1998, 2006, Labandeira et al. 2007). Fertilization in lycophytes and pteridophytes is achieved by sperm in a similar way as in bryophytes, without any known role of animals.

A critical question is whether animal-mediated fertilization in mosses has arisen as an independent parallel to pollination in seed plants or if these phenomena could be historically linked in some way. Such a link could be evolutionary in the sense that structures that promote animal visits have been inherited from algal ancestors or early land plants and modified by selection into homologous structures in bryophytes and vascular plants. Alternatively, the link could be purely functional, meaning that analogous

structures to promote animal-mediated fertilization have developed in different groups, so that animal responses to cues or rewards originally developed by coevolution in one group are secondarily adopted for a similar purpose in another group. This problem thus resembles the long-standing enigmatic questions whether vascular tissue, respectively stomata, are analogous or homologous structures in bryophytes and 'true' vascular plants (lycophytes, pteridophytes and seed plants). However, the development of vascular tissues and stomata, if analogous, have evolved as a response to primarily abiotic selective pressures that are unlikely to be historically linked, whereas the fertilization/pollination processes could well be linked by the presence, at some historic stage, of a common pool of vectors transporting sperm in bryophytes and pollen in seed plants.

In this article I will review possible indications for an ancient origin of animal-mediated bryophyte fertilization and its potential as a precursor of insect pollination syndromes in seed plants, primarily in angiosperms. In this context, I use the term 'precursor' in order to avoid choosing between an analogous or homologous historic relationship between animal-mediated fertilization in bryophytes and insect pollination in angiosperms. I will also point out lines of scientific inquiry that would be needed to test these ideas.

Age of bryophytes versus land-living microarthropods

Bryophytes, springtails and mites are extant representatives of groups of organisms that are thought to have developed and radiated during the early phase of land colonization, although the fossil record is largely absent from the first epochs of this conquest. There is some evidence for a primitive, diminutive embryophyte flora already during the Middle Cambrian in the form of so called cryptospores (Strother 2000). Fragments of land plants originating from the mid-Ordovician period (ca 440–470 million years ago) have been identified as primitive bryophytes (Wellman et al. 2003). With respect to early land arthropods, an extrapolation from the very limited fossil record suggests that Entognatha (including Collembola, Protura and Diplura) and Ectognatha (insects) split during mid-late Silurian by 420 Myr (Engel and Grimaldi 2004). Fossils of both collembolids (Whalley and Jarzembowski 1981) and mites have been found in the deposits of Rhynie Chert, Scotland, which date back to early Devonian, more precisely 411 Myr (Parry et al. 2011). Coprolites (i.e. fossilized fecals from microarthropods) have also been found at Rhynie Chert (Haggood et al. 2003) and other strata of the same age (Edwards et al. 1995, Hagström and Mehlqvist 2012). Detailed studies of coprolites from the Rhynie chert suggest that certain types were produced by collembolids and oribatid mites,

respectively. Fossils of oribatid mites have not been recovered from the Rhynie chert, but they appear in somewhat younger Devonian deposits from Gilboa, USA, dating back to 376–379 Myr (Norton et al. 1988). Thus, according to current knowledge Late Silurian/Early Devonian (cf. Fig. 1) is the earliest documented point in time when microarthropods such as mites and collembolans existed together with bryophytes (and early vascular plants).

Origin of animal-mediated fertilization

Animal-mediated pollination in angiosperms involves cues that attract vectors to the floral structures, rewards that motivate vectors visits to floral structures and mechanisms that promote transfer of pollen. To understand the origin of animal-mediated fertilization in early land plants we need to identify corresponding functions in fossil plants as well as their present-day relatives. The knowledge about such functions in fossil or extant representatives is rudimentary, so this section is by necessity speculative.

Both mites and springtails are richly equipped with morphological structures, such as setae, spines and depressions on the exoskeleton, which could temporarily attach sperm. They live in moist habitats where after terrestrialisation it is necessary to have a hydrophobic exoskeleton in

order to avoid drowning. It is known that sperm masses in mosses are released together with lipids (Paolillo 1979), which reduce the surface tension, and this reduction of the surface tension also enhances the dispersion of sperm (Muggoch and Walton 1942). Thus, it is possible that the hydrophobic properties of the sperm masses serve to attach sperm or sperm mother cells to the microarthropod exoskeleton, thereby promoting a passive transport of sperm.

Microarthropods may also use lipids or other nutrients in sperm masses as a complementary source of food, but these are only available in connection with the male sexual structures and cannot explain attraction to female sexual structures. Notably, analogues to sperm masses are lacking in charophyte algae, since the sperm are released from sperm mother cells that do not separate from the gametophyte (Graham and Wilcox 2000).

Already in 1884 the German botanist Wilhelm Pfeffer (Pfeffer 1884) described that sperm from various plants, such as stoneworts, bryophytes and ferns, were attracted by and actively tracking gradients of substances released from the archegonia or oogonia. In the case of stoneworts, he did not identify any active attractant among the substances he exposed to the sperm. The sexual pheromones in stoneworts or the other sister group to land plants, *Coleochaete*, are still unidentified, but it is known that oogonia of *Co-*

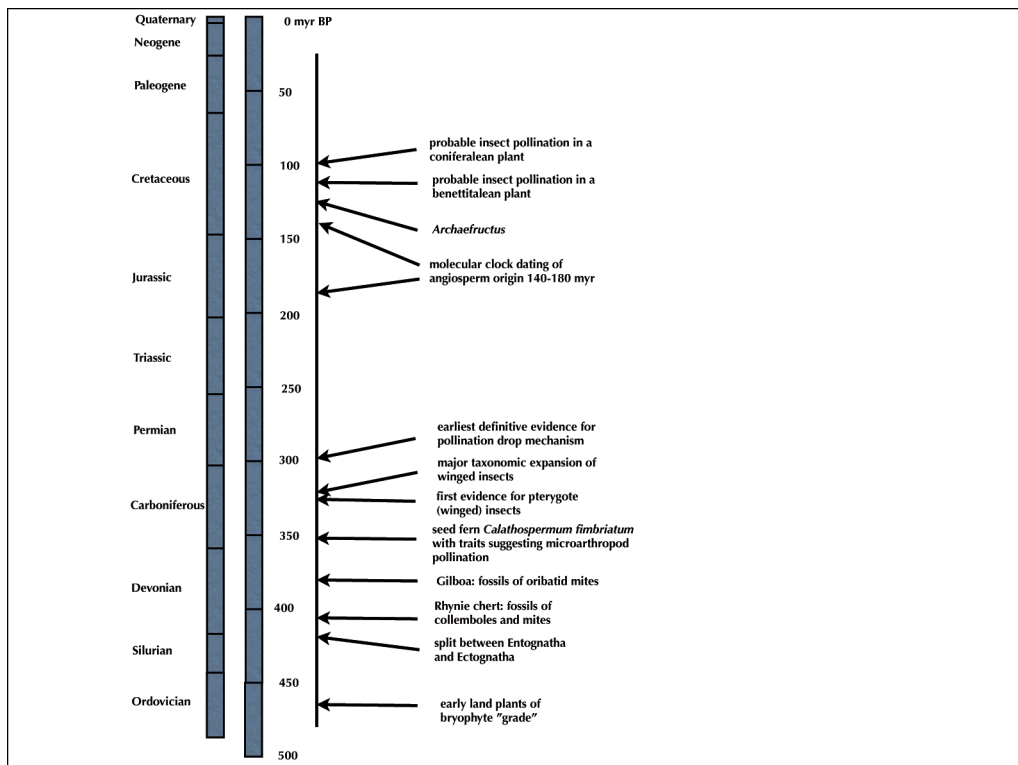


Figure 1. Timeline for important events related to animal-mediated fertilization in bryophytes and insect pollination of vascular plants.

leochaete exude cytoplasmatic contents that are believed to attract sperm (Graham and Wilcox 2000). However, much more is known about sex pheromones in an earlier divergent representative of the charophyte lineage, *Closterium* (Sekimoto 2005), in which several glycoproteins have been identified as agents controlling different stages of its sexual cycle. In the case of mosses, Pfeffer (1884) could specify that the attractant substance was sucrose and that the mechanism seemed to be unspecific across species. Much later, Kaiser et al. (1985) have confirmed that a cocktail of sugars dominated by sucrose is accumulating in the archegonial neck-canal cells of *Bryum capillare* during maturation. These sugars are released when the neck-canal cells disintegrate prior to fertilization (Ziegler et al. 1988). Pfeffer (1884) failed to identify the active signal substance in the liverworts he tested (*Marchantia polymorpha* and *Radula complanata*), although he noted that the two species apparently shared the same attractant. In any case, sugar and other substances released from archegonia or associated structures could potentially attract microarthropods and serve as a reward in bryophyte-animal fertilization syndromes. For example, mucilage is exuded by the paraphyses, sterile structures that surround both antheridia and archegonia. This mucilage is generally considered to assist in keeping the sex organs moist, but some observations suggest that it also attracts various arthropods that use it for food (Harvey-Gibson and Miller-Brown 1927). Another type of attractants were recently identified by Rosenstiel et al. (2012) who demonstrated that springtails are attracted by volatile substances emitted from the moss *Ceratodon purpureus*, comparable to floral scents in angiosperms. The identities and sources of these substances is still unknown, since the volatiles were derived from complete plants including both fertile and sterile parts of males respectively females.

Pre-angiosperm evidence of insect-mediated pollination

So called pollination drop mechanisms occur in all four extant groups of gymnosperms (Gnetophyta, Pinophyta, Ginkgophyta and Cycadophyta; Labandeira et al. 2007). Ovules with one or two outer protective integuments open into a small channel called micropyle. Inside the micropyle is a pollination chamber where the fertilization process is initiated. A drop of fluid is exuded to the outside of the micropyle and this drop traps pollen. The pollination drop is subsequently either resorbed or evaporated so that pollen are transported into the pollination chamber. Pollination drops are functional components of female structures both among extant insect-pollinated (Gnetophyta, Cycadophyta) and wind-pollinated (Pinophyta, Ginkgophyta) gymnosperm groups. Chemical characterization of pollination drops have revealed that they contain sugar as well as amino acids and the concentration of sugar

varies among gymnosperm groups with comparatively low concentration in wind-pollinated conifers and much higher concentration in insect-pollinated gnetophytes (Labandeira et al. 2007). The key question is thus whether pollination drop mechanisms first developed as a means to serve wind-mediated or animal-mediated pollination. The earliest definitive fossil evidence for a pollination drop mechanism is the seed fern *Callospermation pusillum* that lived ca 300 Myr ago during the Late Carboniferous (Rothwell 1977).

The evolution of wings is clearly a key innovation for the development of insect pollination syndromes when it comes to larger, tree-forming plants. The first pterygote (winged) insects – attributed to Archaeorthoptera – have been found from strata dating back to the Carboniferous, ca 324 Myr (Prokop et al. 2005) and a major taxonomic expansion of pterygote insects took place shortly thereafter (by 320 Myr; Brauckmann et al. 1995). According to Labandeira (1998) elongated feeding structures probably adapted for feeding on surface liquids were present in insects already during the Permian, although it is unclear what sort of liquids they may have ingested. These insects however went extinct by the end of the Permian (Labandeira 1998). Several indirect lines of evidence suggest that entomophily developed among gymnosperms during the preangiospermous Mesozoic, including mouthpart construction of insects, presence of pollen types consistent with entomophily and signs of herbivory of reproductive structures (Labandeira et al. 2007). Insect groups such as beetles, mecoptoroids and small diptera have been pointed out as the most probable vectors (Labandeira et al. 2007, Ollerton and Coulthard 2009, Ren et. al 2009). Circumstantial fossil evidence suggests, that insect pollination in cycads may have occurred already during the Middle Triassic (Klavins et al. 2005). Firmer evidence for insect-mediated pollination among gymnosperms does not occur until the Cretaceous and is thus overlapping in time with the angiosperm radiation. The first two well-documented cases of probable insect pollination (Labandeira et al. 2007) involve a coniferalean plant, *Frenelopsis alata* (ovuliferous cone taxon)/*Alvinia bohémica* (microsporangiate cone taxon) from the earliest Late Cretaceous and a benettitalean plant, *Cycadeiodes dactotensis* from the upper Early Cretaceous. These two plants appear to have had quite different pollination syndromes, involving long proboscis flies and pollen-drop feeding in the first case and herbivorous beetle larvae consuming reproductive structures, thereby transporting pollen within a closed strobilus in the second case.

Age of angiosperms

The age of angiosperms is still highly controversial. Age estimates based on molecular clocks differ widely dependent on the various methodological approaches employed

in the analyses: Bell et al. (2005) arrived at 180–140 Myr (Middle Jurassic–Early Cretaceous), Bell et al. (2010) obtained estimates of 199–167 Myr (Early–Late Jurassic) while Smith et al. (2010) suggested 257–182 Myr (Early–Late Triassic). These estimates predate the earliest unequivocal macrofossils (e.g. *Archaeofructus*; ca 125 Myr). There is no clear picture of the phylogenetic relationships among different groups of ancestral seed plants and therefore no firm basis for conjectures about the evolution of floral structures of angiosperms such as carpels, stamens and flowers from the quite different reproductive organs in the other seed plants (Friis et al. 2006). Currently, the only generally accepted fossil evidence for angiosperms predating the Cretaceous is presented by scattered occurrences of angiosperm-type pollen, but this does not give any further information about the plants (Friis et al. 2006). On the other hand, much information has been gained from so called mesofossils, i.e. diminutive fragments of flowers and fruits that have been recovered from strata at several sites in North America and Europe from the mid-Early Cretaceous to the end of the Cretaceous (Friis et al. 2006). These strata have revealed diversified mesofossil floras and, surprisingly, demonstrated that already in the Early Cretaceous all the major basal angiosperm evolutionary lineages such as ANITA-grade (Amborellaceae + Nymphaeaceae + Illiciaceae + Trimeniaceae + Austrobaileyaceae), monocots, magnoliids and basal eudicots were present (Friis et al. 2006). Thus, the first period of diversification of angiosperms is still a black box, and all guesses about their morphology, reproduction, adaptation and environmental requirement must be based on studies of either mesophyll floras or the representatives of basal lineages that have survived until present-day. It should be noted that representatives of lineages with pollination drop mechanisms such as Benetitales and Gnetales often occur together with angiosperm mesofossils (Friis et al. 2009).

Alternative origin of angiosperm pollination

If a fertilization system involving microarthropods and bryophytes was present at the advent of angiosperms, these microarthropods could have expanded from bryophyte fertilization to angiosperm pollination, in analogy with the shifts of pollination syndromes that have been frequently documented among angiosperms. Alternatively, the first pollinators could have been microarthropods preadapted for feeding on spores that changed habit to feed on pollen, which are structurally similar to spores. Such scenarios would reasonably assume that the first angiosperms were herbaceous plants of small stature having diminutive inflorescences, similar in size and shape of bryophyte sexual structures. They must have been growing in fairly moist habitats alongside with bryophytes.

In fact, the first unequivocal fossils of angiosperms, derived from mesofossil strata, have reproductive structures that are small, typically a few mm or less in length (Friis and Skarby 1981, Crane et al. 1995, Friis et al. 2006, 2011) and thus comparable in size to reproductive structures of bryophytes. In the Early Cretaceous two main types of fossil flowers have been recognized in terms of perianth development: 1) naked flowers that lacked perianth, possibly protected by floral bracts, and 2) flowers with perianth and undifferentiated tepals (Friis et al. 2006). The gynoecium consisted of a single carpel, with a poorly developed stigmatic surface. Ovule production per flower appears to have been low. The stamens were very small, with only little sterile tissue between and above the pollen sacs (Friis et al. 2011), which in fact suggests them to have been visually similar to bryophyte antheridia. Nectaries were lacking and it is assumed that pollen was the chief reward to pollinators (Friis et al. 2011). However, there are indications that the androecia may have been involved in the attraction of pollinators. For example, the connective is frequently swollen and sometimes glandular in Early Cretaceous fossils, similar to homologous structures in extant angiosperms (Friis et al. 2006, 2011).

In these cases the actual size of the whole plants is unknown, although the mesofossils are often preserved together with larger fragments of conifer wood, cones and twigs (Friis et al. 2006). A few whole-plant fossils, such as the famous *Archaeofructus* (Sun et al. 1998), were small plants of a size up to a few decimetres. A majority of extant representatives of the ANITA clade, notably *Amborella trichopoda* from the monotypic family Amborellaceae, and species of the family Hydatellaceae, still have small flowers, similar in size to bryophyte inflorescences, suggesting that this is a plesiomorphic trait. The larger size of for example *Nymphaea* flowers is considered to be an adaptation to pollination by more modern insects (Feild et al. 2004).

Several lines of evidence indicate that early angiosperms were herbs or small shrubs rather than trees (Taylor and Hickey 1992, Taylor and Hickey 1996, Friis et al. 2006). Based on interpretation of morphological data from fossil and early-divergent contemporary taxa Taylor and Hickey (1996) put forward the 'Paleoherb hypothesis' (1992), later modified and renamed the 'Herbaceous origin hypothesis' (Taylor and Hickey 1996). They proposed that the early angiosperms were adapted to moist and temporarily disturbed habitats such as shorelines of fluvial systems, where they would have been competing for space with ferns rather than other groups of seed plants. According to this hypothesis efficient reproduction in terms of fast fertilization, fast seed production and flexible use of resources in combination with clonal growth were key innovations making the early angiosperms competitive in disturbed shoreline habitats. Beside arguments based on morphological characters, several other lines of evidence were presented: fossil examples of Early Cretaceous wood from

angiosperms are rare and small in size, suggesting limited secondary growth. Most fossils of early angiosperms have been recovered in strata that are ecologically associated with wet or moist habitats. The oil product oleanane is a putative angiosperm biomarker, according to these authors, and it is known to show a low frequency of occurrence throughout the Jurassic and Early Cretaceous in the sediments, indicative of a low biomass of angiosperms, with a major increase not before the end of the Late Cretaceous.

In a more recent experimental approach, Feild et al. (2004) collected data on the morphology and physiology of present-day early-divergent angiosperms and analyzed it from a phylogenetic perspective. They reached the conclusion that the common ancestor of contemporary basal angiosperms was adapted to grow in dimly lit, disturbed forest in moist understory or streamside habitats. In contrast to Taylor and Hickey (1996) they proposed that this ancestor was a woody shrub. This difference is partially explained by the recently improved knowledge about the phylogenetic relationships among the basal lineages. In particular, *Amborella trichopoda*, which is a woody shrub, has taken the basal-most position in the phylogenetic tree of angiosperms. The conclusion that the first angiosperms were woody shrubs may be biased if we assume that taxa with a longer life expectancy are those that selectively have been able to persist until today. Taylor and Hickey (1996) proposed that vessels were initially restricted to the roots of perennial herbs. More recently, Royer et al. (2010) suggested another type of support for a weedy habit of early angiosperms when they compared leaf economic traits from fossil angiosperms and gymnosperms, since the estimated leaf mass per area was much lower for the former, consistent with a strategy involving rapid resource acquisition. In any case, there seems to be little evidence for secondary growth or wood formation in the fossil record from the Early Cretaceous, whereas the amount of fossil angiosperm wood increases in the strata from the Late Cretaceous.

Based on the limited early angiosperm flower and pollen fossils, the likewise limited insect fossils and inferences from studies of extant most basal angiosperm lineages, evolutionary biologists have tried to deduce the possible histories of pollination syndromes (Hu et al. 2011).

The major hypothesis states that:

- 1) the first angiosperms were insect pollinated,
- 2) more advanced pollination syndromes developed during the mid Cretaceous,
- 3) wind pollination is derived.

The early pollen sacks were small, so that the pollen production per anther must have been limited (Friis et al. 2006). It is also suggested that pollen grains were initially dry without sticky substances that could cause pollen clumping. This could be an adaptation to ambophilous pollination, i.e. pollination by both wind and insects. It could also be an adaptation to pollination by small in-

sects that cannot carry the weight of clumped pollen. This agrees with studies of extant representatives of the ANITA clade, which suggest that pollination by small diptera is an ancient trait, whereas pollination by beetles, which are able to carry heavier pollen loads, is a derived trait (Feild et al. 2004).

Discussion

It may appear somewhat premature to speculate about the age and possible role of animal-mediated fertilization in bryophytes as a precursor to insect pollination in angiosperms, given that the present evidence for this fertilization syndrome is relying on data from two studies involving a few mosses and two unrelated microarthropods (springtails and oribatid mites). On the other hand, both these microarthropods groups were living side by side with the first land plants at least as long ago as during the Late Silurian. Furthermore, the outline of possible evolutionary scenarios could suggest new directions of scientific inquiry or the reevaluation of existing data. For example, it is important to test whether similar fertilization syndromes occur in other major groups of mosses or in the two other bryophyte phyla, liverworts and hornmosses. The hitherto studied mosses have a dioicous breeding system and it would be interesting to compare the situation in monoicous mosses as well. To complete the picture we also need to know whether animals are involved in the sperm transfer of lycophytes and pteridophytes.

When scanning through the literature dealing with the origins of plant–animal interaction and pollination it is somewhat frustrating to note that bryophytes have completely been left out. For example, Labandeira (2006) discusses four phases of plant–arthropod associations in deep time without mentioning bryophytes at all. As a non-specialist it is difficult to evaluate whether the general lack of information about fossil remnants of bryophytes in accounts focussing on land plant radiation is explained by bryophytes being absent from the communities, being present but not fossilized or being present, but overlooked or neglected. Present-day distribution patterns show that bryophytes are in general more diverse and important for vegetation in cool and moist climates (Ignatov and Afonina 1992, Prendergast et al. 1993). Bryophytes are therefore likely to have been more prolific during cooler epochs in the past as well. Data on Phanerozoic climate change (Scotese 2012) suggest that the Late Ordovician–Early Silurian, when the initial radiation of bryophyte groups occurred, was a comparatively cool period in the history of earth. It is notable that bryophytes as well as springtails and mites – groups with roots from this period – represent the best adapted organisms to survive cryoclimatic conditions and that these adaptations may thus have arisen early in their evolutionary history.

Fertilization mediated by animals could potentially have originated by 410 Myr BP, ca 280 Myr before the radiation of angiosperms. During conquest of land, sex pheromones attracting sperm to female reproductive structures involving complex specific molecules in charophytes have been replaced by a chemically much simpler and unspecific system involving sucrose in mosses and some still unidentified substance in liverworts. The amounts of substances released appear to have increased. Maybe, sucrose released from archegonia is also taken up and used as an extra energy source for sperm in the challenging terrestrial environment. In any case, it is possible to envisage that this source of energy could have triggered early microarthropods to visit moss archegonia. The concentration of sucrose exuded from the archegonia of *Bryum capillare* is, however, low (0.086%; Ziegler et al. 1988) compared to the concentrations in nectaries of insect-pollinated flowering plants. Although the concentrations are probably increased by evaporation since it is possible to observe formation of crystallized exudates at the top of old archegonia, the total amounts of sugar released from individual archegonia is likely to be small and only significant for small microarthropods, such as mites, springtails and small Diptera. This may be one reason why complex fertilization syndromes involving large insects have (as far as we know today) not developed in bryophytes.

A key issue is to find mechanisms that trigger microarthropods to move in the direction from males to females. Animal-mediated fertilization would be enhanced if antheridia mature earlier or over a longer period than the archegonia, so that animals are prone to visit the males in the first place and the females thereafter. In fact, for most modern mosses the antheridia mature earlier and over a longer period than the archegonia (Lackner 1939), although both protogyny and protandry is known (Stark 2002). Lackner (1939) provided synoptic phenological patterns for moss species in Germany and among 96 species studied 82% initiated antheridia before archegonia (with later overlap), while 17% initiated antheridia and archegonia simultaneously. Rosenstiel et al. (2012) demonstrated that female mosses of *Ceratodon purpureus* emit a more diverse array of volatile substances than males. They also found a stronger attraction of springtails to females compared to males, suggesting a mechanism for movement in the direction towards females.

Given a possible ancient origin of animal-mediated fertilization in bryophytes, an expansion of microarthropods from bryophyte fertilization to vascular plant pollination could have taken place even before winged insects had developed. A candidate for this is suggested by fossil remains of an ancient seed fern, *Calathospermum fimbriatum* from the Early Carboniferous (ca 352 Myr ago) reconstructed by Retallack and Dilcher (1988). This fossil is especially compelling because several traits suggest that it seem to have been pollinated by animals during a period when all other contemporary seed ferns show adaptations for

pollination by wind. The prepollen was larger (104 μm) than expected for wind-pollinated plants; the ovules were borne inside cupules and the ovules as well as the interior of the cupules were furnished with numerous glandular hairs, which may have offered a nutritional reward to animals or served as guides for visiting animals. The size of the plant is uncertain, but the longest stem fragment is 17 cm. It appears to have been an early successional colonizer along banks of gullies or temporary creeks, i.e. habitats where we would expect bryophytes to also occur. Retallack and Dilcher (1988) suggest that the pollinators could have been canopy-dwelling spiders and mites since winged insects had not developed by that time. It is apparent from the reconstruction that the pollinators must have been small, in the size of few millimeters, in order to penetrate the long and narrow extensions of the ovular integuments. The phylogeny of seed ferns is currently unresolved and it is therefore impossible to speculate about the relationship of *Calathospermum* and plants with true pollen drop mechanisms or stronger proofs for insect pollination. However, its construction points to the possibility that pollination drops originally were an adaptation to animal pollination mediated by wingless arthropods.

It is thus possible to envisage that small organisms belonging to soil-dwelling microarthropod communities subsequently have expanded from fertilization of bryophytes to transfer of prepollen or pollen in spermatophyte lineages. A consequence of such a scenario is that it involves small animals with a feeding behaviour primarily involving ingestion of small algae, fungal hyphi and possibly moss tissue such as protonemata. The consumption of substances released from bryophyte sexual structures could be assumed to have been an optional source of food since these structures are likely to have been available during a limited period and unlikely to provide full nutrition. This is in opposition with the hypothesis of an origin for animal-mediated pollination syndromes involving primarily pollinivorous insects. Spores and pollen have similar properties with respect to size, nutrient content and cell wall material of decay-resistant sporopollenin. It is therefore possible to envisage a link between sporivory and pollinivory. Observations of spores from various early land plants in coprolites show that spores were actually eaten by early microarthropods (Edwards et al. 1995, Habgood et al. 2003, Hagström and Mehlqvist 2012). At present, it is unclear whether these finds represent true sporivory by specialized herbivores or result from accidental spore ingestion by detritivores feeding on litter. It is also uncertain whether the feeders actually gained any nutrients from spores since the spore walls in the coprolites in general appear unbroken (Edwards et al. 1995). Later pollinivorous herbivores developed feeding mechanisms that destroy the cell walls of pollen. In any case, it seems unlikely that the spores as such would have served as an attractant by female bryophytes (or lycophytes and pteridophytes) during fertilization since the production of gametes and spores is

separated in time and space in extant representatives and most likely also in ancestral lineages. In bryophytes the mature sporophytes are usually not present during the fertilization period and they are separated from the sexual structures by the seta.

During the Late Jurassic–Early Cretaceous, when angiosperms are thought to have originated, the climate was again cooling down leading to a ‘mild ice house’ world (Scotese 2012), which would logically indicate expansion of bryophytes. Nevertheless, most of the fossil reference sites from this period appear to have had tropic or subtropical climates. Today, fluvial shores and beds of both lowland and montane tropical forests are prominent habitats for bryophytes (Pócs 1982). Whether this was also the case during the Late Jurassic–Early Cretaceous is uncertain. Ferns are the only other plants that commonly are found together with the angiosperms at some of the best studied fossil formations from the Early Cretaceous (Royer et al. 2010). For example, Miller and Hickey (2008) only recovered a few poorly preserved specimens of liverwort origin, probably belonging to Marchantiales, in otherwise fairly well preserved strata from the Winthrop Formation of Albion–Early Cretaceous age. The mesofossil plant material is preserved as charcoal, suggesting that the sites have been subject to fire and therefore periodically fairly dry. It seems likely that material from wetter and more typical bryophyte habitats has not been preserved in this way. It is also possible that bryophyte remnants have been largely overlooked or neglected by scientists primarily focussing on angiosperms. On the other hand, fossils of both mosses and marchantioid liverworts have been recovered from Burmese amber from tropical *Metasequoia* forests of Cretaceous origin (ca 90–100 Myr). It should also be noted that the specialists of angiosperm phylogeny are facing the same problem of lacking fossil data to explain the origin and early (pre-Cretaceous) evolution of the angiosperm lineages. A solution to this problem is to infer that the angiosperms arose in ecologically specialized (according to the Herbaceous origin hypothesis) and geographically limited areas where fossilization did not occur or fossils have still not been recovered (Smith et al. 2010, Doyle 2012).

Friis et al. (2011) state that it is likely that insect pollination first developed in non-angiosperm seed plants as Bennetitales, Gnetales and cycads, so that early angiosperms may have partially co-opted pollinators from pre-existing pollinator relationships that were already well established. If we accept the Herbaceous origin hypothesis of angiosperms and assume that bryophytes were present in the same moist and disturbed habitats, it is logical to imagine that these plant communities were also populated by soil-dwelling microarthropods and early insects, such as small Diptera. Under such a scenario it is possible to propose the existence of a series of related fertilization/pollination syndromes, which are driven by reward in the form of sugar from archegonia in bryophytes, from pol-

lination drop mechanisms in Gnetales and Bennetitales and from floral structures in angiosperms. We can call this the ‘Bryophyte precursor hypothesis of plant pollination’. Note that I use the term ‘precursor’ in order to avoid choosing if a historic relationship between these syndromes is purely functional (analogous) or linked by evolutionary descent (homologous). Under this hypothesis the animals were small and needed only small amounts of nutrients for their metabolism. They may have been driven by olfactory senses, having siphonous mouthparts or suction feeding behaviour and capacity to carry only small loads such as sperm and small, unsticky pollen. They used the rewards provided by the plants as a secondary source of food, being primarily herbivores, fungivores or detritivores, at least in juvenile stages.

In order to test this hypothesis much more information is needed about the microarthropods and insects that existed together with the first angiosperms, Gnetales and Bennetitales. We also need to confirm that bryophytes occurred in these communities. With respect to the present-day relationships, we need more evidence for animal-mediated fertilization of different groups of bryophytes and characterization of the array of vectors involved, their behaviour and the cues that trigger them to visit sexual structures of bryophytes. Potential associations between bryophytes and more advanced soil-dwelling insects such as grasshoppers and ants could suggest an expansion of the pool of vectors beyond the microarthropods that we studied in the Science article. It is especially compelling that several lines of evidence point out small Diptera as early pollinators. Similar cases of associations between small Diptera and various bryophyte groups would therefore provide additional support for a common historic origin of the fertilization processes. In the near future it may also be possible to test the possibility of an evolutionary link by searching for homologous genes in angiosperms and bryophytes that are controlling rewards and cues related to animal-mediated fertilization/pollination.

At the current state of knowledge it is impossible to give a clear answer to the initial question whether animal-mediated fertilization of bryophytes is a parallel or a precursor to pollination of angiosperms. Modern reconstructions of ancient biota seem to suggest that animal-mediated fertilization has occurred in several contexts involving different groups of plants and animals. Due to the fragmentary fossil data it is difficult to discern whether these contexts have a historic connection or represents cases of parallel evolution. As I see it, a historic link between animal-mediated fertilization in bryophytes and insect pollination in angiosperms is a clear possibility and there is no strong evidence against it. Therefore, the Bryophyte precursor hypothesis of plant pollination deserves to be seriously tested.

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