Microarthropods Mediate Sperm Transfer in Mosses

Nils Cronberg,[1]* Rayna Natcheva,[1,2] Katarina Hedlund[1]

The algal ancestors to all land plants were fertilized by sperm, which could freely swim between male and female structures in the aquatic environment. Some extant land plants, such as ferns, lycopods, horsetails, and bryophytes (mosses, liverworts, and hornworts), are still fertilized by sperm, whereas gymnosperms and angiosperms are fertilized by pollen, which is drought resistant and dispersed by wind or animals. Sperm are usually considered inefficient and poorly adapted to terrestrial conditions because of their dependency on a continuous water layer for dispersal.

We designed a greenhouse experiment to test whether springtails or mites could mediate fertilization between spatially separated male and female mosses. Patches of male and female plants of a cosmopolitan unisexual moss (*Bryum argenteum* Hedwig) were positioned at three different distances, 0 cm (i.e., united), 2 cm, and 4 cm apart, in separate transparent plastic vials (Fig. 1A). A bottom layer of water-absorbing plaster of Paris served as a physical barrier for sperm. This design was replicated (n = 7) for three different treatments: (i) with actively moving springtails (*Isotoma caerulea* Bourlet), (ii) with slower moving oribatid mites [*Scutovertex minutus* (C. L. Koch) and *S. sculptus* Michael], and (iii) without animals. Successful fertilization was expected to result in production of sporophytes physically attached to the mother shoots.

After 3 months, abundant sporophytes were found in vials in which male and female patches were united, where sperm could swim freely (Fig. 1B). No fertilization was observed in the treatment without animals and where the sexes were kept apart at 2 and 4 cm, confirming that sperm were unable to disperse on their own. Numerous sporophytes were produced when animals were present and when moss patches were spatially separated, demonstrating that both springtails and mites were capable of transporting sperm across both distances. The means of this transport is an open question, but presumably the sperm adhere to arthropod cuticle somehow. The results also confirm that distances of sperm transfer between mosses are related to the mobility of the animals, because the test with the more-mobile springtails gave a higher fertilization success at a greater distance.

These observations raise the question of whether animal-mediated fertilization results from passive random movements or active visits to fertile structures, similar to visits of pollinators to flowers. To test this, we conducted a series of preference experiments. In separate sets of vials, we allowed animals to choose between male fertile versus sterile shoots, between female fertile versus sterile shoots, and also between fertile shoots of both genders. Both springtails and mites preferred fertile to sterile shoots (Fig. 1C). We do not know the reason for the attraction, but it may be because fertile shoots are a source of food, because they not only secrete sucrose (*1*) but also starch, fatty acids, and mucilage (*2*–*4*).

Our results suggest that a mutualistic relationship exists between bryophytes and microarthropods. About 50% of moss species are unisexual, having male and female structures on different individuals, which means that fertilization success is distance-dependent and often limited by the availability of mates. When water is scarce and a continuous water film is lacking, animal-mediated sperm transfer seems to be the only possible mode of fertilization, even in bisexual and potentially self-compatible species.

The origin of animal-mediated fertilization has been assumed to involve angiosperms and insects (insect pollination), although angiosperms first emerged during the early Cretaceous (circa 140 million years ago), and some of the insect groups involved in pollination appear to have radiated well before this period (*5*). It is hypothesized that insect pollination started as pollinivory (pollen consumption) and then evolved toward more-complex mutualistic relationships (*6*). Mosses, springtails, and mites are extant representatives of taxa that originated after the early phase of land colonization (circa 440 to 470 million years ago). Animal-mediated fertilization in mosses therefore potentially antedates similar syndromes in other plant groups.

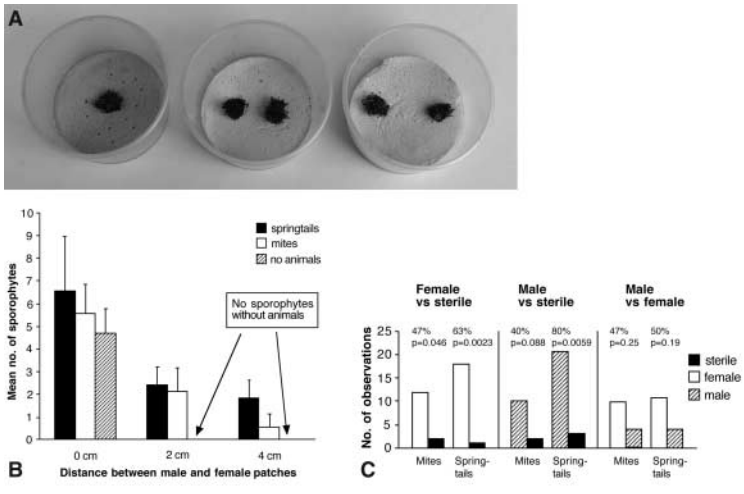

Fig. 1. Fertilization of moss shoots by mites and springtails. (**A**) Vials with male and female moss patches united or separated by 2 and 4 cm. (**B**) Sporophyte production in female moss patches in presence versus absence of springtails or mites. Fertilization was achieved when patches of different sexes were united and, when spatially separated, exclusively in the presence of animals. Each bar represents the mean number of sporophytes in seven replicates (error bars represent one standard error). (**C**) Preference experiment (two-choice test) in which mites and springtails were allowed to choose between fertile and sterile moss shoots. Percentages represent the proportion of 30 replicates in which animals were present on the moss shoots. Bars show the numbers of animals present on fertile or sterile shoots. Significance levels of G tests are also given.

References and Notes

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Supporting Online Material

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Materials and Methods
Tables S1 and S2

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[1]Department of Ecology, Lund University, S-223 62 Lund, Sweden. [2]Bulgarian Academy of Sciences, 23 G. Bonchev Street, 1113 Sofia, Bulgaria.

*To whom correspondence should be addressed. E-mail: Nils.Cronberg@ekol.lu.se