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$$Si + SiO_2 \Leftrightarrow 2\ SiO.$$

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Dinosaur Coprolites and the Early Evolution of Grasses and Grazers

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Silicified plant tissues (phytoliths) preserved in Late Cretaceous coprolites from India show that at least five taxa from extant grass (Poaceae) subclades were present on the Indian subcontinent during the latest Cretaceous. This taxonomic diversity suggests that crown-group Poaceae had diversified and spread in Gondwana before India became geographically isolated. Other phytoliths extracted from the coprolites (from dicotyledons, conifers, and palms) suggest that the suspected dung producers (titanosaur sauropods) fed indiscriminately on a wide range of plants. These data also make plausible the hypothesis that gondwanatherian mammals with hypsodont cheek teeth were grazers.

Today, grasses (in the family Poaceae) are extant on all continents except Antarctica, and numerous organisms, not least humans, depend on them for food (such as cereals and feed for domesticated animals) and habitat (*1*). The absence of an early fossil record of grasses has prevented detailed examination of their evolution and coevolution with animals. Presumed grass pollen (*Monoporites*) in the Maastrichtian to Paleocene [70 to 60 million years ago (Ma)] of South America, India, and North Africa marks the earliest fossil record of Poaceae (*1*, *2*), and unequivocal macrofossils of crown-group Poaceae appear no earlier than the Late Paleocene (~55 Ma) (*2*, *3*). Rare macrofossils and a phytolith record from North America point to diversification of the two main grass subclades, BEP (Bambusoideae + Ehrhartoideae + Pooideae) and PACCAD (Panicoideae + Arundinoideae + Chloridoideae + Centothecoideae + Aristidoideae + Danthonioideae), in the Late Eocene (~35 Ma) (*4*). In contrast, recent molecular clock estimates provide substantially older dates for the origin of both crown-group Poaceae (~83 Ma) (*5*) and of the BEP and PACCAD subclades (~55 Ma) (*3*). Despite these discrepancies, molecules and fossils both support a
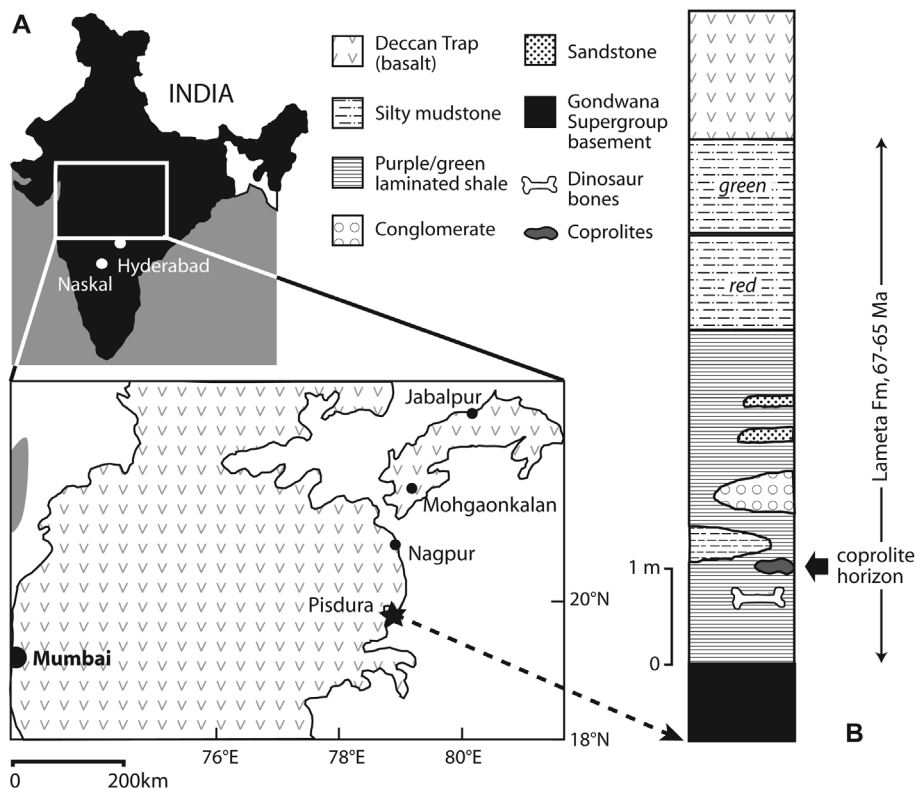
Fig. 1. (**A**) Geographic location of the coprolite site at Pisdura in the Deccan Traps, India. Grass macrofossils have been found at Mohgaonkalan (*18–20*), and an unnamed gondwanatherian mammal has been described from Naskal (small map at top left) (*8*). (**B**) Stratigraphic position of the coprolite horizon in the Lameta Formation in the measured section at Pisdura. The Maastrichtian age of the Intertrappean beds at Pisdura is based on biostratigraphical, magnetostratigraphical, and geochronological data from this and correlated sections (*13*, *14*, *29–31*).

predominantly Cenozoic radiation of the grass clade (*3*). The biogeographical history of grasses is equally obscure. However, the distribution of extant Poaceae taxa point to a South American origin for the family (*3*), an inference that is supported by the aforementioned earliest occurrences of grass pollen (*2*). Current molecular dating and fossil data (*3*, *5*) also favor long-distance dispersal of all major Poaceae lineages after the Late Cretaceous breakup of Gondwana.

Because grasses were thought to have been rare in pre-Cenozoic ecosystems, they have not been considered as food for Late Cretaceous herbivores (*2*, *6*). Titanosaur sauropods, the most prominent terrestrial plant-eaters in Gondwana, do not display any dental features (such as grinding cheek teeth) that would point to grass-eating (*6*, *7*). Mammals with typical grazing adaptations, particularly high-crowned (hypsodont) teeth, occur mainly during the Oligocene and Miocene, indicating that grasses were not previously present in sufficient abundance to form major parts of an herbivorous diet (*2*). A striking exception are the enigmatic sudamericid gondwanatherians, known from the Late Cretaceous of South America, Madagascar, possibly Tanzania, and India, as well as the Paleogene of South America and Antarctica [reviewed in (*8–10*)]. Their highly hypsodont teeth appear decidedly suited for handling abrasive materials, such as grass (*11*). However, because of the lack of contemporary grass fossils, they have been interpreted as an adaptation to a semiaquatic or burrowing lifestyle, reminiscent of that of modern beavers (*11*).

Here we provide fossil data on Late Cretaceous grasses that shed light on the early evolution of grasses and grass-eaters. The grass fossils, in the form of phytoliths, are preserved in coprolites from the Late Cretaceous (Maastrichtian) Intertrappean beds of the fluvio-lacustrine Lameta Formation, at Pisdura in central India (Fig. 1). The coprolites are found in abundance weathering out on surface exposures (*12*). The four types (A, B, Ba, and C) of coprolites have all been ascribed to titanosaur sauropods, based on their common association with titanosaur skeletal remains (*13–15*). Previous work on type A coprolites shows that they contain remains of predominantly C$_3$ plants, including conifers and cycads, but also bacterial colonies, fungal spores, and algal remains (*14*, *15*).

We extracted bio-opal (phytoliths, diatoms, etc.) from all types (A, B, Ba, and C) of coprolites (*16*). The recovered organic and silicified plant fossils include cells and tissue from what were likely (i) dicotyledons [polyhedral and anticlinal epidermis, often with vesicular

infillings, helical tracheary elements, honeycomb mesophyll, cystoliths, and nongrass trichomes (*17*)]; (ii) large pieces of silicified parenchymatous tissue probably referable to conifers; (iii) rare globular echinate phytoliths diagnostic of palms; and (iv) numerous silica phytoliths of certain-to-likely grass origin. The range of grass morphotypes is similar to that found in phytolith assemblages extracted from the leaves of modern grasses. To taxonomically place the fossil phytoliths, we collected information on Poaceae epidermal anatomy and grass silica short cell (GSSC) morphology from the literature and a

phytolith reference collection (table S1) (*17*) and mapped these data onto a cladogram of the grass family (*1*) (fig. S1).

The phytolith data from the coprolites reveal a diversity of phytolith morphotypes (expressed as new phytolith morphotype genera and species; see supporting online text), suggesting that several grass taxa representing different Poaceae subclades were extant in India during the Late Cretaceous (Figs. 2 and 3). These include: (i) a derived ehrhartoid (*Matleyites indium*), (ii) possibly another grass within the [Bambusoideae + Ehrhartoideae]
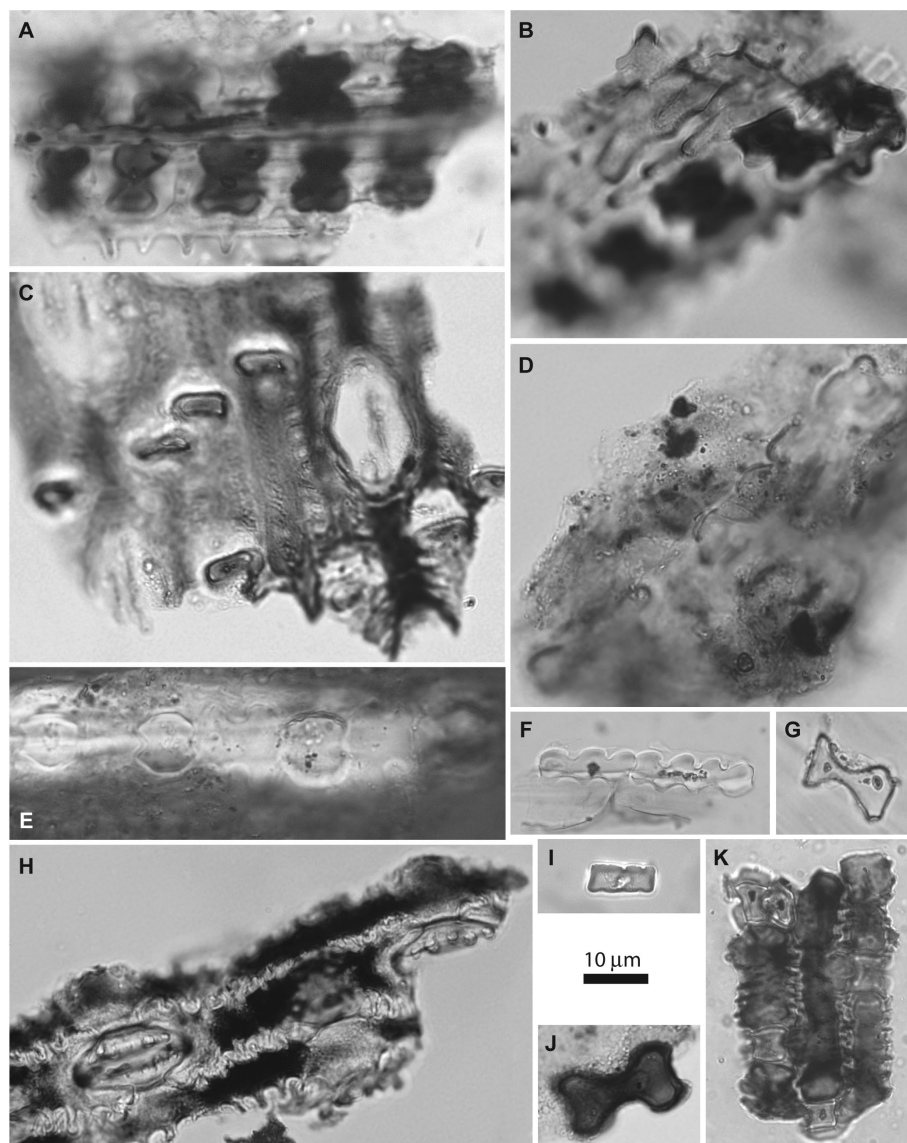

Fig. 2. Selected fossil phytolith morphotypes described here. (**A** and **B**) *Matleyites indium*. Costal row(s) of vertical "oryzoid" bilobate and cross-shaped GSSCs with pointed lobes associated with papillate epidermis. (**C**) *Jainium pisdurensis*. Intercostal epidermis with stomates and vertical chusquoid/bilobate GSSCs. (**D**) *Chitaleya deccana*. Costal row (obliquely across the photo) of three symmetrical bilobate and polylobate GSSCs with concave ends. (**E**) *Pipernoa pearsalla*. Costal row of collapsed saddles. (**F**) *Chitaleya deccana*. Two polylobate GSSCs in a row. (**G**) *Eliasundo lameti*. Isolated tabular angular bilobate GSSC. (**H**) *Vonhueneites papillosum*. Fusiform stomates with papillate subsidiary cells associated with papillate epidermal long cells. (**I**) *Thomassonites sinuatum*. Isolated trapeziform sinuate ("crenate"). (**J**) *Matleyites indium?* Isolated bilobate GSSC with pointed lobes. (**K**) *Stebbinsana intertrappea*. Epidermis with horizontal trapezoidal bilobate GSSCs with concave top. A light microscope was used to take these images [in (E), with a Nomarski filter].

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clade (*Vonhueneites papillosum*), (iii) a grass within the [Bambusoideae + Ehrhartoideae] clade or the Puelioideae (*Pipernoa pearsalla*), (iv) a grass of the PACCAD clade or with affinity to Pooideae (*Chitaleya deccana*), (v) an ingroup pooid (or PACCAD grass) (*Thomassonites sinuatum*), and (vi) another grass with affinity to PACCADs (*Eliasundo lameti*). Several other grass morphotypes (*Jainium pisdurensis* and *Stebbinsana intertrappea*) attest to the morphological diversity of GSSCs, but their systematic position within Poaceae cannot be ascertained. Although some of the phytolith morphotypes may derive from the same grass, a minimum of five different taxa from the [Bambusoideae + Ehrhartoideae] clade and the

PACCAD or Pooideae clades are represented (supporting online text). Thus, most of the morphotypes observed appear to be more derived than the basal-most grass taxa (*Anomochloa*, *Streptochaeta*, and *Pharus*), based on GSSC morphology and the assumption that these ingroup taxa can confidently be used to inform plesiomorphic character states within Poaceae. The existence of crown-group Poaceae in Late Cretaceous India is consistent with the presence there of Intertrappean macrofossils described as pooids (*18–20*) (Fig. 1) and the occurrence of grass pollen (*Graminidites*) in the Lameta Formation of the Nand-Dongargaon Area (*21*).

The presence of ehrhartoids and possible ingroup pooids in the Late Cretaceous shows

that the BEP clade had diversified substantially by this time (Fig. 3), much earlier than had been thought on the basis of fossils and molecular clock dating (*1, 3, 4*). An early radiation of both the Pooideae and the PACCAD clade is also suggested by the occurrence of GSSCs typical of pooids and PACCAD grasses, respectively. It is not yet clear whether the ecomorphologies that allow extant pooids and PACCADs to inhabit open, arid habitats had evolved by the Late Cretaceous.

The hypothesis that extant grass subgroups arose in Asia and subsequently spread to Gondwana by way of India by the Late Cretaceous is highly improbable in light of the data pointing to a collision between the Indian subcontinent and Asia in the Paleogene (55 to 50 Ma), with no compelling support for an earlier connection (*22, 23*). The diversification of the BEP and PACCAD clades could have occurred on the Indian subcontinent, after which grass taxa dispersed globally via Asia and North America, starting in the Paleocene. This option is unlikely, given the paucity of grass pollen in Paleocene-Eocene floras of the Northern Hemisphere as compared to the Southern Hemisphere (*2*). Our preferred hypothesis is that the various Poaceae subclades had spread in Gondwana by the Late Cretaceous, as a result of either vicariance or dispersal [for example, via the Kerguelen Plateau or the Gunnerus Ridge (*24, 25*)]. This scenario does not necessitate a Gondwanan origin of Poaceae or any of its subclades; the initial diversification of extant lineages could have occurred in India, in another Gondwanan continent, or in the Northern Hemisphere [compare to discussion in (*10*)]. However, it postulates that BEP and PACCAD grasses ranged across at least part of Gondwana by the time India lost its biogeographic connections with other southern continents; that is, by ~80 Ma (*22, 24, 25*).

The examined phytoliths show variable preservation, implying that some phytoliths (such as large pieces of tissue) were incorporated into the coprolites as part of foodstuffs, whereas others (such as rare palm phytoliths) may have derived from soil stuck to ingested plant material. Previous studies of coprolite and enterolite material from Late Cretaceous herbivorous dinosaurs have suggested that these animals fed mainly on conifers but also consumed cycads and angiosperms (*14, 26, 27*). Phytolith data show that their diet must have been very mixed, including conifers, cycads (*14*), dicotyledons, and grasses. Because phytolith production in extant grasses is magnitudes higher than in dicotyledons and conifers, the relatively low abundance of grass phytoliths in the coprolites may indicate that grasses did not form a major part of the animals' diet.

There is as yet no direct evidence for mammalian grazing in the Late Cretaceous. However, the presence of both diverse silica-rich grasses and sudamericid gondwanatherians in
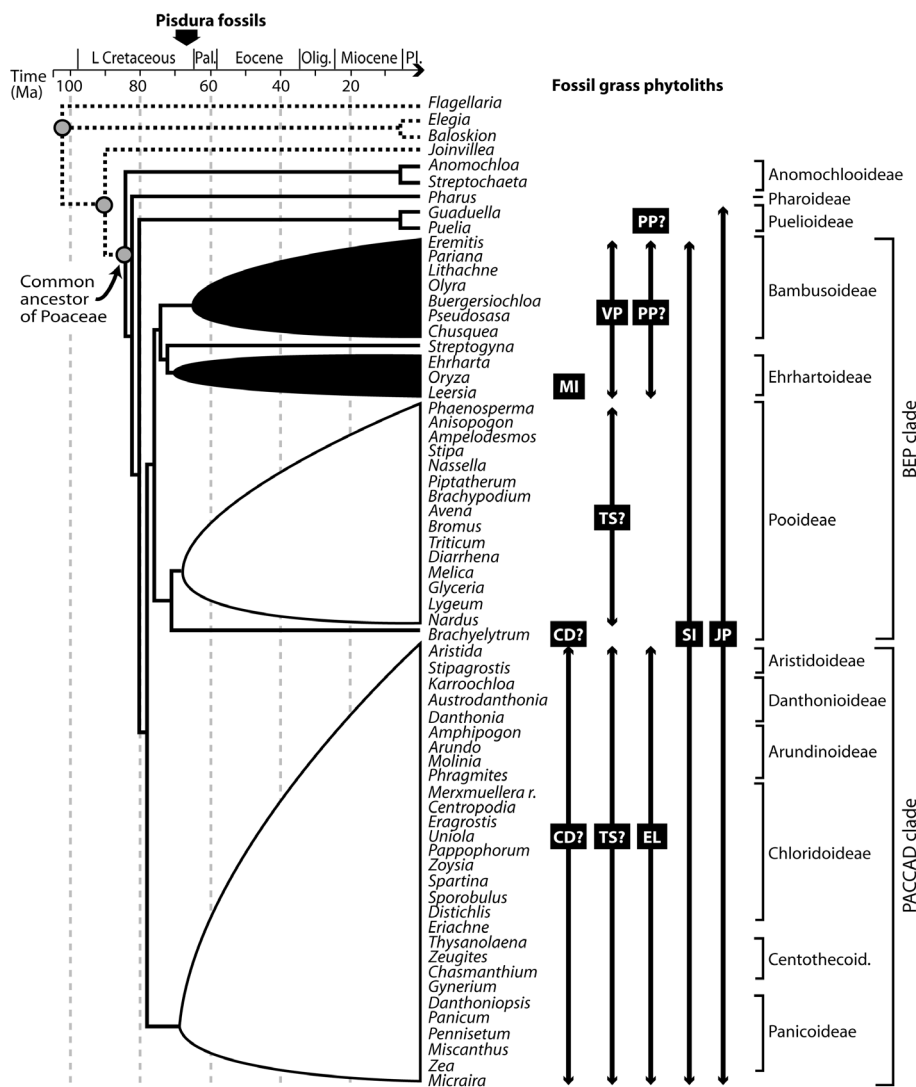

Fig. 3. Systematic affinities of fossil phytolith morphotypes (Supporting Online Text) reported from Maastrichtian coprolites from Pisdura, central India, suggesting significantly older dates for taxonomic diversification within the grass family (Poaceae) than previously assumed (*1–3*). The phylogeny is from the Grass Phylogeny Working Group (*1*). Approximate ages for the crown node of Poaceae and for immediate sister taxa (marked with a gray circle) were provided by molecular clock analysis (*5*). White shapes indicate open-habitat grass clades; black shapes and all other terminal taxa indicate closed-habitat grasses. Fossil phytolith morphotypes are as follows: CD, *Chitaleya deccana*; EL, *Eliasundo lameti*; JP, *Jainium pisdurensis*; MI, *Matleyites indium*; PP, *Pipernoa pearsalla*; SI, *Stebbinsana intertrappea*; TS, *Thomassonites sinuatum*; VP, *Vonhueneites papillosum*.

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the Intertrappean beds of India makes it plausible that hypsodonty in these animals was an adaptation to feeding on abrasive grasses. Moreover, the phytolith data suggest that silica production in grasses comparable with that observed in extant taxa appeared to have evolved by the Late Cretaceous. This rejects the view that modern levels of phytolith production were an evolutionary response to grazing during the Cenozoic (28) and suggests that the high silica levels of grasses are the result of coevolution with Late Cretaceous herbivores (such as gondwanatherians or insects) or of a process unrelated to plant/herbivore interaction.

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Pre- and Postinvasion Defenses Both Contribute to Nonhost Resistance in *Arabidopsis*

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Nonhost resistance describes the immunity of an entire plant species against nonadapted pathogen species. We report that *Arabidopsis* PEN2 restricts pathogen entry of two ascomycete powdery mildew fungi that in nature colonize grass and pea species. The PEN2 glycosyl hydrolase localizes to peroxisomes and acts as a component of an inducible preinvasion resistance mechanism. Postinvasion fungal growth is blocked by a separate resistance layer requiring the EDS1-PAD4-SAG101 signaling complex, which is known to function in basal and resistance (*R*) gene–triggered immunity. Concurrent impairment of pre- and postinvasion resistance renders *Arabidopsis* a host for both nonadapted fungi.

Host cell entry represents a critical step during pathogenesis of invasive animal and plant parasites (*1*, *2*). This is usually not a barrier to adapted phytopathogenic fungi that are able to infect a plant species. However, in plant species beyond the host range of a pathogen, called nonhost plants, parasitic fungi typically fail to enter attacked plant cells (*3*, *4*). In the nonhost interaction between *Arabidopsis* and the grass powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*), three *Arabidopsis pen* (penetration) mutant loci were recovered that permit, at high frequency, entry of the nonadapted parasite (*5*), thereby providing initial evidence for the existence of a plant-controlled process terminating fungal ingress at the cell periphery. *AtPEN1* encodes a soluble NSF (*N*-ethylmaleimide–sensitive factor) attachment protein receptor (SNARE)–domain-containing and plasma-membrane resident syntaxin, which becomes recruited into plasma membrane microdomains beneath incipient fungal entry sites (*6*, *7*). Because SNARE proteins play a key role in vesicle trafficking in eukaryotic cells (*8*), these findings have been interpreted as evidence for the existence of a vesicle-associated resistance mechanism preventing powdery mildew ingress.

Although each of the isolated *pen* mutants (*pen1*, *pen2*, and *pen3*) permits efficient *Bgh* entry, initiation of postinvasive fungal growth invariably ceases, and this coincides with a cell death response of epidermal cells with haustorial complexes (*5*). We performed a time-course experiment and compared *Bgh* entry rates in wild type, *pen1*-1, *pen2*-1, and *pen1 pen2* double null mutants (Fig. 1). Entry rates were seven- and fivefold higher than wild type in *pen1* and *pen2* mutants, respectively. An 11-fold increase over wild type was seen in the *pen1 pen2* genotype, suggesting *PEN1* and *PEN2* act in separate defense pathways (Fig. 1). Elevated fungal entry rates were associated with an increased incidence of invasion-associated cell death (Fig. 1). To assess the importance of *PEN1* and *PEN2* functions in a further powdery mildew nonhost interaction, we examined the mutant *Arabidopsis* genotypes with *Erysiphe pisi*, which colonizes dicotyledonous pea plants in nature. Like *Bgh*, *E. pisi* fails to reproduce on *Arabidopsis* but

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