

investigations of entire microbial communities (i.e., microbiomes). This microbiome revolution has led to a more refined understanding of host–microbe interactions, which indicates that the impact of microbes can depend on overall community composition or interactions among species, host phenotype and physiology, or location within the host [8–10]. Thus, it is critical to place our knowledge of the biology of potentially bSTMs into the broader context of the entire microbial community, as well as consider host–microbe and microbe–microbe interactions that might influence microbial function and subsequent fitness consequences.

Third, the evolutionary significance of bSTMs may be contingent on factors that promote the evolution of microbe–host cooperation, such as mode of transmission and reproductive mode. Current examples of bSTMs are known from pea aphids, mosquitoes, humans, and fungi [4]. In all cases, however, the microbe exhibits mixed-mode transmission (i.e., horizontal and vertical transmission), which is likely to alter the evolutionary landscape for both host and microbe. Moreover, in the pea aphid, a series of asexual (parthenogenetic) generations is followed by a single generation of sexually produced offspring. We thus caution against interpreting the aphid work as broadly generalisable, especially as related to bSTMs being a benefit of multiple mating to females.

Fourth, while we welcome suggestions for future tests of the bSTM hypothesis, these approaches will require clear predictions and protocols. For example, we raised the idea that females may preferentially eject ejaculates when they have high microbial loads, we did not predict, as Lombardo *et al.* [2] stated, that ‘females should be able to retain or expel semen based on whether it contains bSTMs or pSTMs’, although this is not necessarily implausible (see [11] for evidence of a similar process in the mouse

colon). Tests of the latter idea, however, will need to control for microbial load. Similarly, Lombardo *et al.* [2] proposed an experiment testing whether females experimentally inoculated with pSTMs seek copulations outside of their fertile periods as a particularly strong test of the bSTM hypothesis. This is problematic: the notion of a female fertile period is rather misleading in several taxa, for example, those with prolonged female sperm storage, such as birds [12], and induced ovulators. In addition, while the term inoculation is never defined, such intervention must provide the female with appropriate stimuli associated with mating and insemination in order to elicit the predicted response.

To conclude, we thank Lombardo *et al.* [2] for their discussion, and encourage studies examining host fitness consequences of STMs and their implications for sexual selection, sexual conflict, and the evolution of multiple mating, placed into an appropriately nuanced framework of reproductive microbiomes [1], and with clear, testable predictions.

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## Forum

### Threshing Yards: Graveyard of Maternally Borne Seed Microbiome?

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**Plant domestication, at least in cereals, is associated with the loss of the shattering allele. In such species, grains are manually harvested and threshed, leaving behind naked seeds. This could have led to the loss of maternally borne seed microbiomes and their associated benefits in modern-day domesticated species.**

#### Domestication Syndrome and the Microbiome

Archaeological and phytolith evidence indicates that the domestication of crop plants from their wild relatives was under way sometime between 8000 and 5500 BP, followed closely by the development of agricultural tools between

7000 and 4000 BP [1]. During this period, scores of plants were domesticated for enhanced productivity through inadvertent or directional selection. Hundreds and thousands of crop generations later, the domesticated species began to differ from their wild ancestors in a suite of traits, often referred to as the 'syndrome of domestication' [2]. The syndrome includes a range of traits preferred by humans [2]. Here we argue that one of these changes – namely, the loss of dispersibility due to loss of the shattering gene – could have had a potentially devastating consequence of depriving modern-day crops of their maternally borne seed microbiome. This could potentially explain the observed differences in the seed microbiome between wild and domesticated crop species [3].

### Loss of Shattering Gene and the Threshing Process

The extraordinary success of grasses, indicated by their widespread occurrence and dominance, is partly due to their mechanism of dispersal. Grass fruits (seeds) can be transmitted by animals or wind. Unlike in other angiosperms, grass fruits are dispersed with parts of their flower or even inflorescence attached, referred to as diaspore. The diaspore aids in the dispersal of the fruit and establishment of the seedling [4]. In a grass inflorescence, the zone of abscission could be below the fruit, pedicel, rachilla, or branch [5] and determines whether the fruit is dispersed alone or as a unit in packaging. After analyzing 250 grass taxa, Yu *et al.* [6] concluded that the most likely position of the abscission zone in ancestral grasses was inside the spikelet; thus, fruits were dispersed along with surrounding floral tissues. Although this zone has shifted to the pedicels or to inflorescence branches, it is rarely located below individual fruits [5].

In all domesticated grass species, there is a conspicuous loss of grain shattering. Thus, seeds or grains are retained on the plants, without loss due to dispersal. In

*Oryza sativa* (rice) and *Sorghum bicolor* (sorghum) the disappearance of the shattering trait was due to the loss of the alleles *qSH1* and *sh4*, and *SpWRKY*, respectively [7]. These alleles contribute to the formation of the abscission layer in the pedicel or rachilla of the wild species. This has led to the development of manual or mechanical threshing processes to remove the grains from domesticated cereals and millets (Figure 1). The latter process cleanses the seeds of all of the seed accessory structures that would have normally accompanied the seed during dispersal and seed germination. In short, the threshing yards delivered naked seeds, bereft of the associated maternal structures.

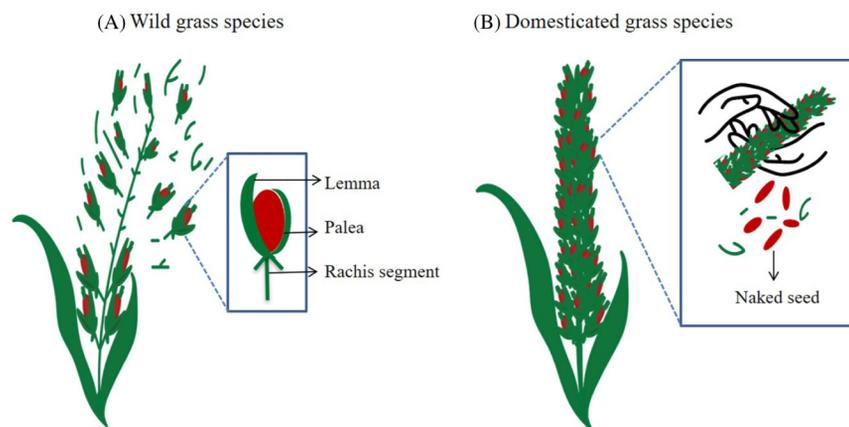
### Consequences of the Loss of Seed Accessory Tissues for the Seed Microbiome

Surprisingly, there is little literature addressing the consequences of the loss of seed accessory tissues in the modern-day seed. Although the primary role of these tissues is mechanical protection of the developing seed and aiding in dispersal [8], they could also contribute to the maternal microbiome to the seeds. The seed microbiome plays an important

role in numerous plant growth processes, from seed germination to protection of seeds and seedlings from pest and diseases. Their loss therefore could have several adverse consequences for plant growth and adaptation [8].

There is some evidence that seeds of several species, cereals included, contain a microbiome, presumably stocked while the seed was developing on the plant [8]. It is obvious that much of this native microbiome would be the first port of call for the germinating seed before it acquires a more contemporaneous microbiome from its new habitat. Thus, the microbiome of the newly emerging seedling would comprise a certain maternal memory while adapting to the new environment. In this context, it is easy to visualize how the various seed structures that once went along with the seed could have provisioned the seed microbiome community as the plant began its next generation.

We propose that the seed accessory structures that accompanied the seed in the wild species could have served as reservoirs of a maternal pool of microbiota for the seed and next-generation seedling. This is supported



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Figure 1. Contrasting Fruit Dispersal Features in Wild and Domesticated Grass Species.

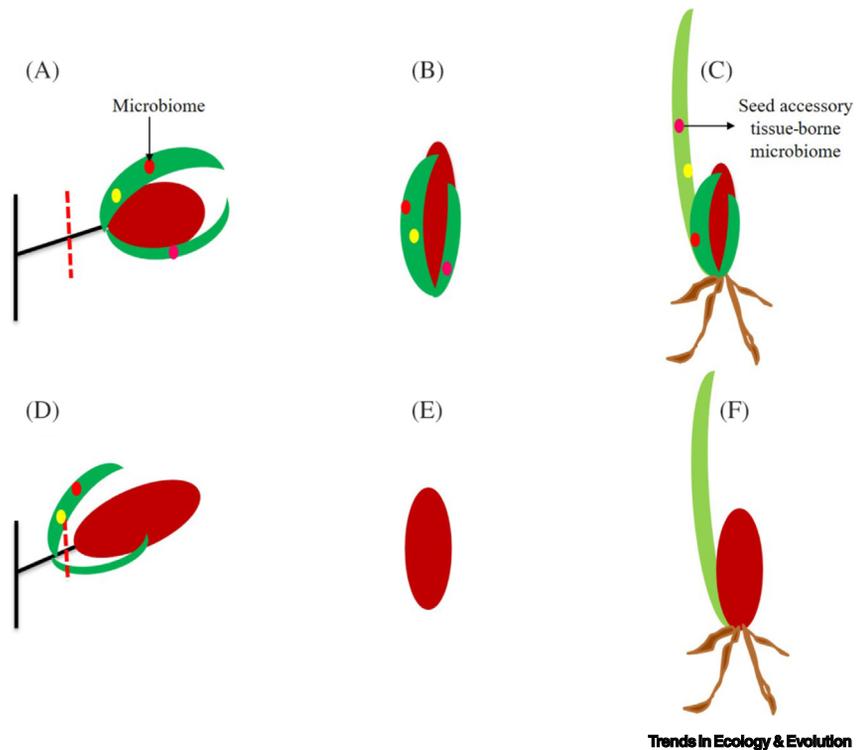
For a Figure360 author presentation of Figure 1, see the figure legend at <https://doi.org/10.1016/j.tree.2020.08.010>. (A) In wild species, the dispersal unit is a spikelet that shatters at the abscission zone and is dispersed with the seed accessory structures including the lemma and palea and the rachis segment. (B) In the domesticated species where shattering does not occur, seeds are forced out of the plant through manual or machine threshing.

by the finding that plant tissues differ in their endophyte composition, meaning that, at the very least, ‘unique’ endophytes would have been lost along with the seed accessory tissues [9]. Explicit studies have shown the role of awns in the hygroscopic movements and burial of seeds for them to germinate. Such awns could also contribute to enriching the microbiome pool of the seeds. The same is possible with the lemma and palea, which, in addition to their primary function of protecting seeds against desiccation or digestion, could harbor specific microbiota for the seeds (Figure 2).

For example, an analogous situation is that babies delivered through the vaginal canal are endowed with greater microbiome diversity than those born through Cesarean section [10]. Such babies have better neonatal immune system priming than those born through Cesarean section [10]. Similarly, while it is common knowledge that the primary function of the seed coat is to offer protection to the endosperm and embryo within, the seed coat is known to play an equally important role in mobilizing resources to the developing seed [11].

### Research Challenges

We believe that the road ahead is to unequivocally demonstrate: (i) the presence of a microbiome in the seed accessory tissues (some of which could even be unique to the tissues); (ii) that this microbiota is passed on to the next generation of seedlings; and (iii) that the microbiome has a fitness benefit. Ideally, such studies could be conducted in wild relatives of domesticated taxa such as rice, wheat, or sorghum using both culture-dependent and culture-independent metagenomic tools. It is interesting to note in this context that, in barley and wheat, washings of both lemma and palea yielded vastly higher numbers of filamentous fungi and bacteria than did their seeds alone [12]. It would be important to evaluate the role of such seed accessory tissue-borne microbiota in



**Figure 2. Role of Seed Accessory Tissues in Transmission of Microbiome in Wild and Domesticated Crop Species.** (A and B) In wild species, the dispersal unit is the spikelet that shatters from the abscission zone located at the rachis. When the spike is released from the panicle, the seeds are dispersed with the accessory seed structures including the lemma and palea and the rachis segment. (C) As seeds germinate, the microbiome present in the seed accessory structures enters into the growing seedling. (D) In the domesticated species where shattering does not occur, seeds are harvested and threshed. (E) This process cleanses the seed of all accessory structures except the seed coat, leading to what could be referred to as naked seeds. (F) Seedlings growing from naked seeds will be devoid of seed accessory tissue-borne seed microbiome. Broken red lines indicate abscission zones and smaller dots indicate microbiomes.

imparting fitness benefits such as resistance to abiotic and biotic stresses and growth promotion to the seedlings. Affirmation of the hypothesis could lead to a paradigm shift in how we view domesticated taxa and the agronomic processes that are currently used in their cultivation. If, through these studies, we realize that threshing yards are the burial grounds for maternally derived microbiomes, is it not time to exhume them and apply them in the service of the domesticated crops? It is likely that, though this process, we might be able to restore the lost maternal microbiome and their associated fitness benefits to modern-day crops and thereby render them more resilient to abiotic and biotic stresses.

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## Forum

## Functional Genomics Offers New Tests of Speciation Hypotheses

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Speciation is a fundamental process shaping biodiversity. However, existing empirical methods often cannot provide key genetic and functional details required to validate speciation theory. New gene modification technologies can verify the causal functionality of genes

with astonishing accuracy, helping resolve questions about how reproductive isolation evolves during speciation.

## Genomic Tools to Study Speciation

Understanding the process of speciation is one of the main goals of evolutionary biology. Theories concerning speciation are among the best developed in biology (e.g., [1–3]), and throughout the **-omics** (see Glossary) era, expectations have been high for validating theories about how reproductive isolation (RI) evolves to create new species. However, while an abundance of empirical work has raised our understanding of various aspects of speciation [2,3], there is still a paucity of experimental studies that explicitly distinguish alternative hypotheses. This is because many studies lack the fine genetic detail, functional link to RI, and direct manipulation experiments to substantiate theory. Motivated by the dawn of a technological revolution in functional genomics (Box 1), we outline how testing of classical hypotheses in speciation could benefit from advances in gene modification and move beyond current ‘candidate gene validation’ studies, to help spur new research and discovery in the field.

## Pleiotropy and Tight Linkage in Speciation Studies

One of the main goals of speciation research is to ascertain the genetic basis of RI. Modern approaches utilize methods such as **genome scans**, **quantitative trait loci (QTL) mapping**, or **genome-wide association studies (GWAS)** to identify the regions of the genome with high population divergence or association with traits causing RI. However, depending on the frequency of recombination and density of markers, such genomic regions can still contain multiple, sometimes hundreds, of genes and regulatory elements. Such low resolution means that distinguishing effects of **pleiotropy** from those of tight **genetic linkage** of several genes is difficult

## Glossary

**Chromosomal inversion:** a structural rearrangement of DNA sequence where the inverted sequence is reversed relative to the collinear sequence.

**Coupling:** collective effects of different traits or factors involved in reproductive isolation, which strengthen the barrier to gene flow between the diverging populations.

**Dobzhansky–Muller incompatibilities:** negative epistatic interactions between different genes, often in hybrids.

**Ecological speciation:** evolution of RI between populations as a result of ecologically based divergent natural selection.

**Epistasis:** a phenomenon in which the effect of an allele at one locus is dependent on an allele (or alleles) at one or more other loci (i.e., a between-locus interaction).

**Genetic linkage:** a nonrandom association of alleles at different loci. Also, a term used by classical geneticists to refer to genes that reside on the same chromosome.

**Genome scans:** method of comparison between populations/species across the genome to identify differentiated genetic regions across the genome.

**Genome-wide association studies (GWAS):** studies associating genotypic and phenotypic variation, generally using segregating natural genetic variation.

**Heterosis:** hybrid vigor, or a phenomenon of enhanced function of a trait in hybrids.

**Knockdown:** artificial reduction of gene expression by blocking its transcription or breaking down mRNA.

**Knock-in:** artificial insertion of the nucleotide sequence into a genome.

**Knockout:** artificial permanent deactivation of the gene with loss of its functionality.

**Magic traits:** a trait subject to divergent ecological adaptation, which has a pleiotropic effect, causing premating isolation.

**Mutation order speciation:** accumulation of different incompatible mutations in separate populations subject to the same selective regime.

**-Omics:** fields of biology with names ending in -omics and that aim to collect large data sets of biological molecules that translate into the structure, function, and dynamics of an organism. Examples include genomics, transcriptomics, proteomics, or metabolomics.

**One-allele mating mechanism:** a scenario when RI forms due to the same allele spreading in both diverging populations (e.g., a single allele that induces assortative mating with a self-referenced phenotype).

**Pleiotropy:** a phenomenon when a gene affects more than one phenotype.

**Quantitative trait loci (QTL) mapping:** a statistical method of associating genetic and phenotypic variation via establishing sets of recombinants via genetic crosses.

**Reproductive isolation (RI):** genetically based barriers to gene flow between populations/species.