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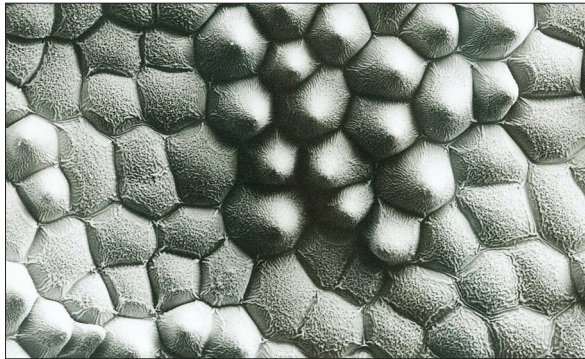
# Beneficial interactions

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# 16

## OM16.1 CHANGES TO CELL SHAPE CAN ENHANCE FLOWER ATTRACTIVENESS AND REPRODUCTIVE SUCCESS

An example of cell shape enhancing attractiveness to insects was identified in flowers of *Antirrhinum majus* (snapdragon). Conical cells are common on flower petals but are uncommon on leaves; in *A. majus* the *MIXTA* gene encodes a MYB family transcription factor necessary for determining conical-shaped cells in petals. **Figure OM16.1-1** illustrates the different appearance of *mixta* mutant flat cells and a patch of conical wild-type cells that arose following localized reversion of the *mixta* mutation. Conical wild-type petal cells confer advantages in attracting insects because: (i) they enhance the petal color by refracting light toward the cell vacuoles where the pigments are; and (ii) they provide a tactile surface that enables bees to come to rest while feeding. Comparing *mixta* mutant flowers with those of wild-type showed that floral attractiveness and plant reproductive success was reduced in the *mixta* mutant. This finding was based on field trials in which wild-type flowers of *A. majus* were found to be more attractive to insects and to set more fruit than *mixta* mutant flowers.



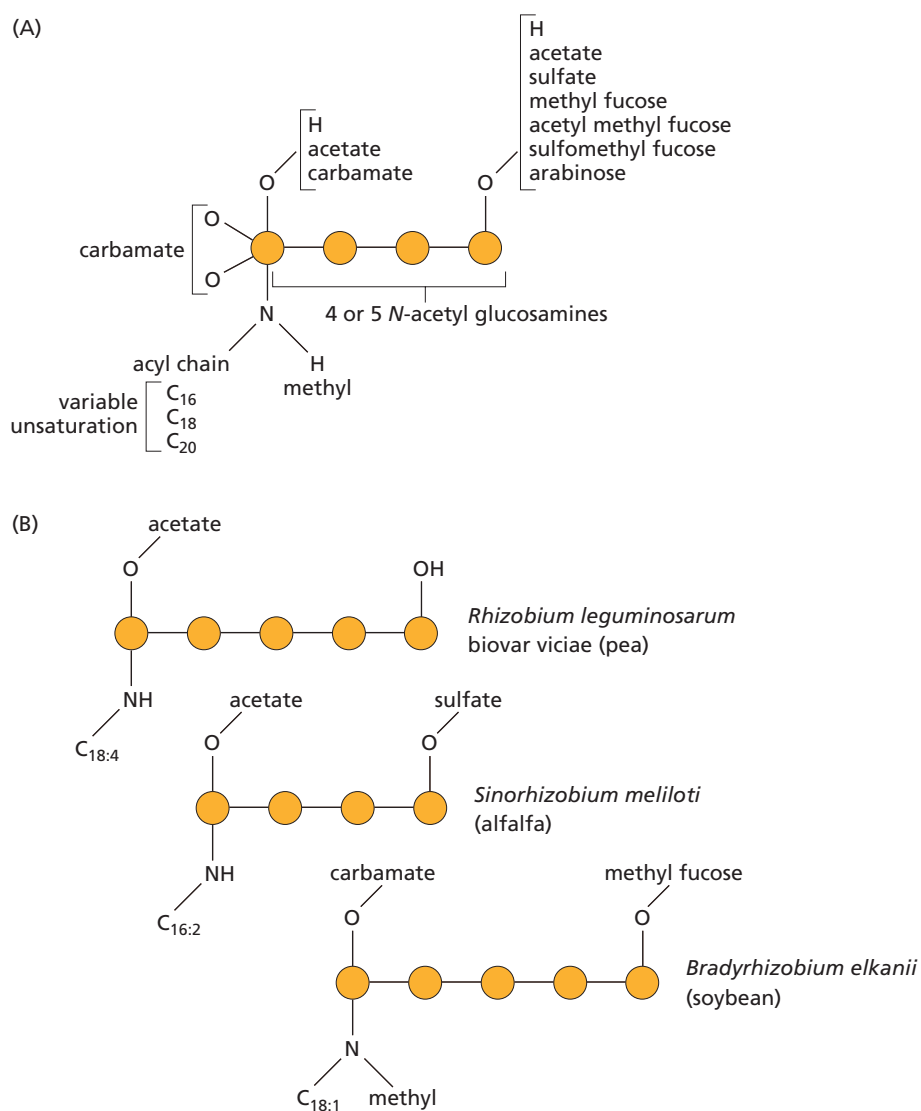
**Figure OM16.1-1 Comparison of conical wild-type cells and *mixta* mutant cells in petals of *Antirrhinum*.** Scanning electron micrograph of the epidermis of an *Antirrhinum majus* petal showing flat-looking *mixta* mutant cells surrounding a patch of conical wild-type cells. The patch of wild-type cells occurred because the *mixta* mutation is caused by a transposon that can excise and restore a wild-type phenotype (see Section 2.2). During petal development such an excision in a *mixta* mutant plant gave rise to a patch of cells in which the wild-type gene, hence conical cell shape, were restored.

## OM16.2 RHIZOBIAL BENEFITS FROM ACQUISITION AND TRANSMISSION OF GENES DETERMINING SYMBIOTIC NITROGEN FIXATION

In addition to improving plant growth, the symbiotic association of rhizobia with legume plants allows the rhizobia to proliferate, and frequently rhizobia in a single nodule (up to  $10^8$ ) are clonal (arising from one infecting bacterium). Thus, even if only 0.001% of rhizobia in a nodule survive and escape after the plant dies, this represents a 1000-fold increase from that single infecting bacterium in a growing season. Therefore, bacteria that have acquired the symbiosis genes can have a strong selective advantage over other bacteria. An example of horizontal gene transfer conferring symbiotic proficiency and a growth advantage on native bacteria was seen when *Lotus corniculatus* was planted in New Zealand with a rhizobial inoculant (treatment with non-native rhizobia known to nodulate *Lotus*): after some years, the bacteria nodulating the plants had retained the original symbiosis genes but were otherwise genetically different from the inoculant strain. A large chromosomal cluster of contiguous genes important for symbiotic nitrogen fixation had been transferred from the bacteria in the original inoculant into indigenous soil bacteria, enabling them to nodulate the introduced legume. Many rhizobial symbiosis genes are in large clusters either on the bacterial chromosome or on large plasmids; they are often referred to as “**symbiosis islands**.” Such clustering of symbiosis genes can facilitate their transfer, even between bacteria that are genetically unrelated to each other. In this way the ability to participate in symbiotic nitrogen fixation can be transferred widely in the bacterial kingdom.

## OM16.3 STRUCTURES AND SPECIFICITY OF SOME Nod FACTORS

Nod factors have a common core structure: a chitin backbone of usually four or five  $\beta$ -1,4-linked *N*-acetyl-D-glucosamine residues, carrying a long acyl chain that replaces the *N*-acetyl group on the terminal sugar (Figure OM16.3-1A). There are many other potential substituents and each rhizobial species makes a specific range of Nod factors. For example, Nod factors from pea-nodulating *Rhizobium leguminosarum* and alfalfa-nodulating *Sinorhizobium meliloti* differ in the structure of the acyl chain and in the presence of a sulfate group on the *S. meliloti* factor (Figure OM16.3-1B). Nod factors from the soybean nodulator *Bradyrhizobium elkanii* have *N*-methyl, carbamate, and methyl fucose substituents (see Figure OM16.3-1B). Different complements of *nod* genes determine the attachment of sulfate and/or acetyl, carbamoyl, methyl, arabinosyl, and fucosyl substituents as well as different fatty acids. The transfer of *nod* genes between rhizobia can change the range of legumes nodulated by a rhizobial strain by changing the Nod-factor structures.



**Figure OM16.3-1 Nodulation signals (Nod factors) produced by rhizobia.**

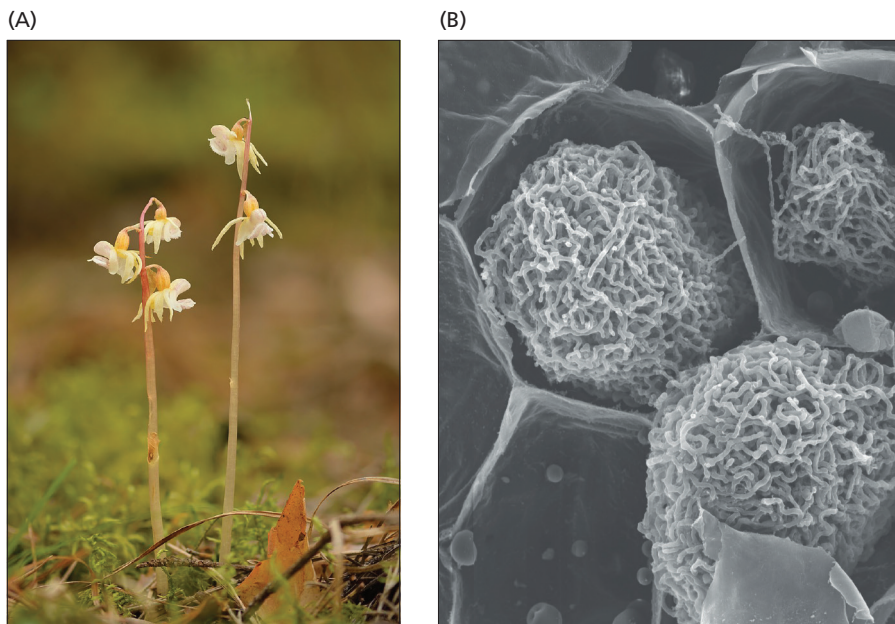
(A) Generic Nod factor showing various types of substituents that can be added by different rhizobial species. The sugar nucleotide UDP-*N*-acetyl-D-glucosamine is polymerized to form a chitin oligomeric backbone of usually four or five  $\beta$ -1,4-linked *N*-acetyl-D-glucosamine residues (*brown circles*). Various *nod* gene products add different substituents to this backbone. Some form of an *N*-linked long acyl chain is always present, but the type of acyl chain (containing 16, 18, or 20 carbon atoms and different numbers (0–4) of unsaturated double bonds) depends on the *nod* genes in a particular rhizobial strain. The attachment of other groups to the chitin backbone also depends on the *nod* genes present in a particular rhizobial species (or strain). (B) Examples of Nod factors made by three different rhizobial strains that nodulate pea, alfalfa, and soybean.

## OM16.4 ORCHIDACEOUS MYCORRHIZAE

One major group of plants – orchids (Orchidaceae) – has an association with soil fungi that differs from those of other angiosperms. After the Asteraceae, the Orchidaceae is the most species-rich family of angiosperms, containing almost 10% of all known angiosperm species. Unlike other angiosperms, orchids growing in natural conditions are completely dependent on mycorrhizal associations (orchidaceous mycorrhizae) for growth beyond initial germination. Orchid seeds are minute and contain insufficient reserves to support seedling growth. After germination, a tiny structure (a protocorm) arising from the embryo forms associations with soil fungi that provide the carbon, nitrogen, phosphate, and other nutrients required for seedling growth and the establishment of photosynthesis. Adult orchids retain mycorrhizal associations, but both the extent to which they obtain nutrition from the fungus and the potential benefits to the fungus vary hugely between species and with environmental conditions. In many species the presence of a mycorrhizal association is not necessary for growth of the adult plant. However about 200 species of adult orchid remain completely dependent on their associated fungi. They contain no chlorophyll, and exist entirely below ground except for the production of an inflorescence (**Figure OM16.4-1A**). This characteristic has arisen about 30 times independently in different orchid genera during orchid evolution.

Orchid roots form mycorrhizal associations with many different species of fungi. A single plant may associate with a single fungal species, or with two or more simultaneously, or with different fungal partners depending on environmental conditions. Many fungal partners are basidiomycetes that live in the soil as **saprophytes** – organisms that obtain their nutrition from dead organic material. The nutrient supply to orchids during germination and to non-photosynthetic orchids thus comes largely from the products of degradation of soil organic matter, transported via hyphae into the roots. Some species of fungi that form orchidaceous mycorrhizae also form ectotrophic mycorrhizae, so it is possible – particularly in the case of non-photosynthetic orchids – that fungi may provide the orchid with carbon obtained from a local ectotrophic mycorrhizal association.

The hyphae of orchidaceous mycorrhizal fungi penetrate the cell walls of the root cortex of their hosts, and ramify inside the cells to form knitting wool-like balls called **pelotons** that may occupy most of the cell volume (**Figure OM16.4-1B**). The fungus remains outside the plasma membrane of the host, which becomes enormously invaginated to envelop each individual hypha and provide a very large surface area over which nutrient transfer can occur. The invasion of cortical cells is accompanied by extensive changes in gene expression in the host, indicative of up-regulation of many transporters, some pathways of primary metabolism, lipid metabolism, and vesicle transport.



**Figure OM16.4-1 Orchidaceous mycorrhizae.** (A) A non-photosynthetic orchid, *Epipogium aphyllum* (European ghost orchid). This species is found in woodland and is dependent on various basidiomycete fungi that also form ectomycorrhizal associations with nearby trees. (B) Pelotons in root cells of *Dipodium variegatum*, a non-photosynthetic orchid of eastern Australia. (A, from “*Epipogium aphyllum* – Johvi” by Ivar Leidus. Licensed under CC BY SA 4.0 via Wikimedia Commons. B, courtesy of Liz Kabanoff.)

The peloton is a relatively short-lived structure, persisting for a few weeks or months before being destroyed by host enzymes capable of attacking fungal cell walls, probably including glycoside hydrolases and chitinases. The expression of genes for these enzymes is up-regulated in roots containing pelotons. The nature of host-mediated destruction of the fungus is a matter of debate. Like endotrophic mycorrhizae, orchidaceous mycorrhizae are probably able to invade host cells because they are specifically recognized by the host and are thus able to evade host defense mechanisms. It may be that specific recognition fails over time, and host defenses are triggered leading to hyphal destruction. It has also been speculated that degradation of pelotons specifically provides the root cells with a large source of nutrition. It is technically very challenging to dissect the precise dynamics of the fungus–host relationship in orchids growing in natural conditions, and much remains to be understood about this phenomenon.