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Plant development: How competing modes of growth coexist in close proximity

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A new study in *Citrus* reveals how CENTRORADIALIS prevents axillary buds from terminating as thorns by directly inhibiting *THORN IDENTITY1*, thereby maintaining coexisting states of determinate and indeterminate growth at vegetative nodes.

In plants, shoot meristems, the source of stem cells, can either terminate growth as structures such as flowers, thorns, and tendrils (determinate growth) or grow indefinitely at the tip or side of the plant as apical and axillary meristems, respectively (indeterminate growth). In many plants these structures and their competing modes of growth coexist in a region between the stem and the base of a leaf (the axil) (Figure 1A). Yet, despite their close proximity on the shoot and their apparent equal distance from developmental cues derived from the leaf and apical meristem, these structures retain distinct developmental fates. It is known, for example, that proximity to the adaxial side of the leaf is necessary for development of axillary meristems[1], but how the balance of determinate and indeterminate growth in such meristems is mediated at close proximity remains a relative mystery. A new study by Zhang *et al.*[2] published in this issue of *Current Biology* brings new insight to these mechanisms by examining how lateral meristems can either terminate as thorns or grow indefinitely as axillary meristems.

In most *Citrus* species, each node along the stem is comprised of a leaf with both a thorn and a dormant bud in its axil. During the course of normal development, this dormant bud can produce either lateral branches or determinate flowers. The mechanisms regulating dormancy of axillary buds are well characterized, with a gene belonging to the TCP gene family, *teosinte branched1*/*BRANCHED1* being a central hub for integrating cues necessary for growth arrest[3,4]. Interestingly, previous work revealed that the orthologs of *BRANCHED1* in *Citrus* — *THORN IDENTITY1* (*TI1*) and *THORN IDENTITY2* (*TI2*) — are necessary for production of thorns, at least in part, by directly repressing a regulator of stem cell identity, *WUSCHEL*[5]. Loss of *TI1* and *TI2* function converts thorns to branches, and releases axillary buds from dormancy, consistent with a derived role in thorn production and conservation of their role in branching.

The finding that *TI1* and *TI2* are necessary for thorn identity yet have a conserved role in growth of axillary buds raises the question: why do branch meristems not develop as thorns but instead remain indeterminate in their growth? To answer this question Zhang *et al.*[2] examined an instance of where the coexistence of determinate and indeterminate growth has been lost. In thornless key limes, two dormant buds exist in the axil of each leaf instead of both a thorn and dormant bud. Transcriptome analysis comparing thorned and thornless key lime shoots identified a *CENTRORADIALIS* homolog (*CsCEN*) that was highly expressed in thornless plants. Loss of *CsCEN* gene function resulted in plants with nearly all nodes having two thorns, one of which was in the position of the dormant bud of wildtype plants. The necessity of *CsCEN* function for the maintenance of indeterminate growth was further confirmed by the loss of *WUSCHEL* expression in the axillary buds of mutant plants. Overexpression of *CsCEN* yielded plants strikingly similar to *ti1 ti2* mutants, where thorns are transformed into branches[5]. These parallels were consistent at the molecular level, as *TI1* expression was reduced in *CsCEN*-overexpressing plants. Thus, *CsCEN* is necessary for the coexistence of determinate and indeterminate states at the leaf axil — too much or too little CsCEN results in the loss of this coexistence.

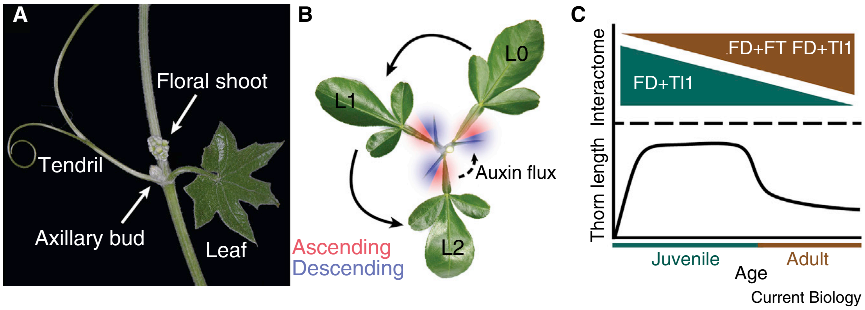

Figure 1. Developmental patterns and potential mechanisms for coexisting modes of growth in leaf axils.
(A) Coexistence of determinate (tendril and floral shoot) and indeterminate (axillary branch/bud) structures in the axil of a species in the Cucurbitaceae. (B) Hypothesized auxin asymmetry in *Citrus*. Solid arrows represent phyllotactic pattern of leaf emergence (for example, L0 to L1 to L2). Thorns and axillary buds in this individual are consistently on the descending and ascending side, respectively, of the phyllotactic pattern (axillary buds not visible). Dashed arrow represents direction of auxin flux from ascending side of preceding leaf to descending side of new leaf. (C) Hypothetical model for how thorn size and presence vary throughout the life cycle of *Citrus*. Age-dependent changes in the suite of proteins capable of interacting might reduce the influence of thorn identity factors during adulthood.

In many passionflower species, tendrils and flowers coexist with axillary buds. In this case, the initiation of the determinate structures precedes the formation of the indeterminate one, suggesting a temporal mechanism to specification of growth fate[6,7]. This mechanism appears less likely in *Citrus*, where Zhang *et al.* previously showed that thorn and axillary meristems co-occur early in their ontogeny[5]. Interestingly, *Citrus* thorns show a consistent arrangement relative to leaves (Figure 1B). Given that spatial variation of the hormone auxin in the shoot apex is known to control the regular initiation of leaves (a pattern known as phyllotaxy), the offset pattern of thorn initiation supports a role for auxin signaling in their initiation[5]. One potential explanation for the coexistence of thorn and dormant bud might be the asymmetries of auxin depletion experienced between descending and ascending sides of the developing leaf primorida[8]. As new leaves initiate at the apex, they act as auxin sinks, pulling more auxin from the side of the preceding primorida closest to them (the ascending side). Do thorns consistently develop on the descending or ascending side within a species? If so, does this auxin asymmetry play a role in determinate and indeterminate modes of growth at axillary nodes? An interesting avenue of future study would be to address if determinate vegetative structures, such as thorns and tendrils, show consistent positioning relative to dormant buds and how this relates to known mechanisms of phyllotaxy.

The asymmetry of thorn and axillary bud growth raises the question as to how CsCEN acts in an asymmetric fashion. How are the effects of CsCEN spatially restricted within the leaf axil? Interestingly, when Zhang *et al.*[2] examined localization of *CsCEN*, they found that it was confined to the developing axillary bud in wild-type plants; in contrast, *TI1* was found only in thorn primordia. However, in plants with loss of *CsCEN* function, *TI1* was also found in the position of the axillary bud, supporting that the asymmetry of *TI1* localization is mediated by the asymmetry of *CsCEN*. Analysis of *FLOWERING LOCUS D* (*CsFD*) — a gene known to work in complex with many members of the PEBP protein family, to which CEN belongs[9,10] — revealed symmetric localization throughout both thorn primordia and axillary bud. Use of a reporter assay in *Citrus* protoplasts revealed that CsCEN in combination with CsFD was capable of repressing *TI1* expression. These results further illustrate that the information for asymmetric regulation of determinate and indeterminate growth is mediated by *CsCEN*.

In many species, a paralog of *CEN*, *TERMINAL FLOWER1*, is important for suppressing the formation of floral meristems[11]. This, in addition to localization in vegetative axils in *Arabidopsis*[12], makes *TFL1* a good candidate for a role in the indeterminate growth of the *Citrus* axillary bud. When Zhang and colleagues targeted *CsTFL* using CRISPR, they found no vegetative phenotypes in *Citrus*[2]. This is not entirely surprising given previous work showing that *CsTFL* is high in floral organs but largely absent in vegetative tissue of *Citrus sinensis*[13]. However, it was also shown that when grown at low, floral-inductive temperature, *CsTFL* abundance becomes detectable and is higher in juvenile than adult stem segments[13]. This likely means that CsTFL works with CsCEN during the juvenile phase to maintain the indeterminate state of axillary buds during periods of floral-inductive stimulus. Without CsTFL, axillary buds might prematurely develop as flowers. It will be interesting to see if the *CsTFL* mutants have a shortened juvenile phase similar to other species. The fact that *CsTFL* plays no discernible role in branching patterns makes it an attractive target for strategies attempting to accelerate flowering time in *Citrus*.

These findings also suggest a mechanism by which vegetative traits become diminished with the onset of reproductive competence[14]. In *Citrus*, the thorns that characterize the juvenile phase are often absent or greatly reduced on flower-bearing adult shoots[15]. Given that TCP proteins interact with both FD and FLOWERING LOCUS T[16,17], it is possible that age-dependent changes in the TI1 or TI2 ‘interactome’[11] result in competition between partners important for thorn and flower development (Figure 1C), thereby reducing the size or presence of thorns, or even the production of floral buds in place of thorns[18,19]. If such a mechanism proves accurate, it will give new insight into the evolution of age-dependent defenses in *Citrus* (and possibly thorny species more broadly). In particular, it raises the possibility that shifts in defense strategies during a plant’s life cycle are a consequence of constraints levied by the competing mechanisms of thorn and flower production, similar to the competing mechanisms that act to maintain the coexistence of determinate and indeterminate growth during the vegetative phase.

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Sex pheromones: Made with a little help from my (bacterial) friends

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Males of the olive fruit fly *Bactrocera dorsalis* team up with *Bacillus* bacteria in their rectal glands to synthesize 2,3,5-trimethylpyrazine (TMP) and 2,3,5,6-tetramethylpyrazine (TTMP) from glucose and threonine. The bacterially produced TMP and TTMP are utilized as sex pheromones to attract virgin females.

Efficient communication is essential for a biologically successful life. However, finding the right medium to communicate is not trivial as most organisms live in a vibrant biotic environment where each individual from the multitude of coexisting species shouts its message out into the world. Luckily, organisms can employ a variety of different acoustic, visual, haptic, gustatory, and olfactory signals. Chemical signals, however, represent the oldest form of communication[1]. Microbes have been using chemical communication since long before complex, multicellular life evolved, and chemical communication is still widely used across the tree of life. In insects it represents the most common way of communication, especially in intraspecific communication[2]. Intraspecific-communication substances are called pheromones[3], regardless of their structure or origin, and they serve diverse functions including attracting conspecifics and mating partners, warning them about dangers, and conveying information about individual identity, directions, or food sources. Retaining a clear communication channel with closely related species amongst the chemical chatter of the animate world represents a challenge. Sharing a long evolutionary history with other species also includes sharing a chemical ‘language’. Thus, young species are under strong selection pressure to modify their chemical vocabulary to ensure specific communication, requiring either signals of unique compounds or a unique blend of compounds. However, evolving novel signals is not necessarily trivial. Although existing signal molecules can be, in principle, easily modified with single novel enzymatic steps[4], their evolvability might be constrained by multiple functions[5] and de novo evolution of completely novel pathways is a highly complex process[6]. Thus, insects have been repeatedly reported to employ an alternative strategy to gain access to novel informative substances: they capitalize on the unique biosynthetic metabolism of microbes that provide an entire arsenal of potential signals novel to the animal world[7]. As a consequence, they often engage in stable (symbiotic) associations and coevolve these pre-existing compounds and their perception into specific signals, or pheromones. A new study by Ren *et al.*[8] published in this issue of *Current Biology* describes such a case: *Bacillus* bacteria harbored in the rectum of the oriental fruit fly *Bactrocera dorsalis* (Tephretidae) are the source of a male sex pheromone containing the two compounds 2,3,5-trimethylpyrazine (TMP) and 2,3,5,6-tetramethylpyrazine (TTMP) (Figure 1).

Both sexes of Tephretid flies are known to employ pheromones to attract the opposite sex, including the male-produced compound TMP[9–11]. Ren *et al.*[8] first conducted a deeper analysis of extracts from mature male recta that proved to attract virgin females. Gas chromatography coupled to mass spectrometry revealed TMP as well as TTMP, 2-ethyl-3,5-dimethylpyrazine, and 2,3,5-trimethyl-6-ethylpyrazine. A test