Commentary

How to be STYLISH: columbine study sheds new light on the obscure mechanisms of nectary formation

'Make analogous research by suppressing the nectaries at different moments. Study their development, learn their parts, discover the manner in which they form their excretion, and its uses.'

(Jean Senebier, 1800, *Physiologie Végétale*)

This urgent plea, made more than two centuries ago by a Genevan naturalist, laid a wonderful framework to start exploring the development, evolution and function of nectaries, one of the most intriguing organs of flowering plants. The role and composition of nectar have been extensively studied; by comparison, the structures responsible for its production have received little attention and a heap of crucial questions remains. Nectaries are incredibly diverse but few studies have examined the mechanisms behind their emergence (Bowman & Smyth, 1999; Lee, 2005; Morel *et al.*, 2018). In this issue of *New Phytologist*, Min *et al.* (pp. 1090–1100) identify the *STYLISH* genes (*STY*) as novel regulators of nectary formation in the Colorado blue columbine (*Aquilegia coerulea*). This study is illuminating and timely. It brings functional information on nectary development in a group that occupies a key phylogenetic position, the so-called basal eudicots. It also coincides with the publication of new findings on *CRABS CLAW*, the only other known regulator of nectary formation (Gross *et al.*, 2018; Morel *et al.*, 2018).

'*This study is illuminating and timely. It brings functional information on nectary development in a group that occupies a key phylogenetic position, the so-called basal eudicots.*'

A nectary by any other name would taste as sweet

In Ancient Greece, néktar was the mythical beverage of gods. It is not clear when the term was first used to describe the sugary liquid produced by flowering plants but this terminological choice suggests that its functional importance was acknowledged very early

This article is a Commentary on Min *et al.*, **221**: 1090–1100.

on. The structure that produces this 'mighty' substance however, remained largely overlooked by taxonomists and morphologists until the end of the nineteenth century (Bernardello, 2007).

Nectaries are extremely labile structures. Their position, number, size, morphology, colour and mode of action vary tremendously (Fig. 1). Floral nectaries can be associated with any organ within a flower. They function mainly in pollinator attraction but occasionally fulfil a protective role. The nectary of tobacco, for instance, protects the ovary from pathogens by secreting a nectar containing proteins with antimicrobial and antifungal properties. Nectaries are also encountered on shoots and leaves in more than 100 families of angiosperms, and even in ferns. These extrafloral nectaries mostly attract predatory insects that act as 'bodyguards' and protect the plants from herbivores. Sporadically, they can lure insect prey (in carnivorous plants) or even guide pollinators to neighbouring flowers (in *Euphorbia* sp.).

Looking for the master regulator(s) of nectaries

Due to their variable nature, a universally accepted definition of nectaries did not emerge instantly. Nectaries are glandular organs, best defined by their function, that is their ability to produce nectar. Some use modified stomata, others use secretory trichomes or simply rely on cell wall rupture in the nectariferous tissue to release their secretion. In columbine, the nectaries are located on the inner surface, at the very tip of the spur-like shaped petal. Yant *et al.* (2015) used a transcriptomic approach on micro-dissected tissues to try and identify regulators of nectar spur formation. Looking for genes specifically expressed in the emerging pouch (future spur), the authors identified a homologue of *STYLISH*, *AqSTY1*, as top hit. The *STY* family encodes zinc-finger transcription factors that control several aspects of plant development, including pistil development across the core eudicots (Gomariz-Fernández *et al.*, 2017). As columbine belongs to the basal eudicots, the function of *AqSTY1* could not be inferred from the literature. Min *et al.* first compared the expression profile of *AqSTY1* and two other members of the *STY* family, *AqSTY2* and *AqLRP*, in the developing bud. In early stages, all three homologues were strongly expressed in the ovules and at the tip of the carpels and petals primordia. As spurs start differentiating, *AqLRP* expression ceases in the petal while *AqSTY2* becomes moderately expressed across the growing pouch and *AqSTY1* transcripts become restricted to the inner side of the expanding spur tip, where nectaries will develop. To gather functional evidence, Min *et al.* employed a virus-induced gene silencing (VIGS) approach to downregulate the expression of *AqSTY1/2* and *AqLRP*. While silencing *AqSTY1* alone did not induce any visible effect, style development was abnormal in 10% of triple knock-down flowers, consistent with the function of *STY* homologues in core eudicots. Strikingly, a third of
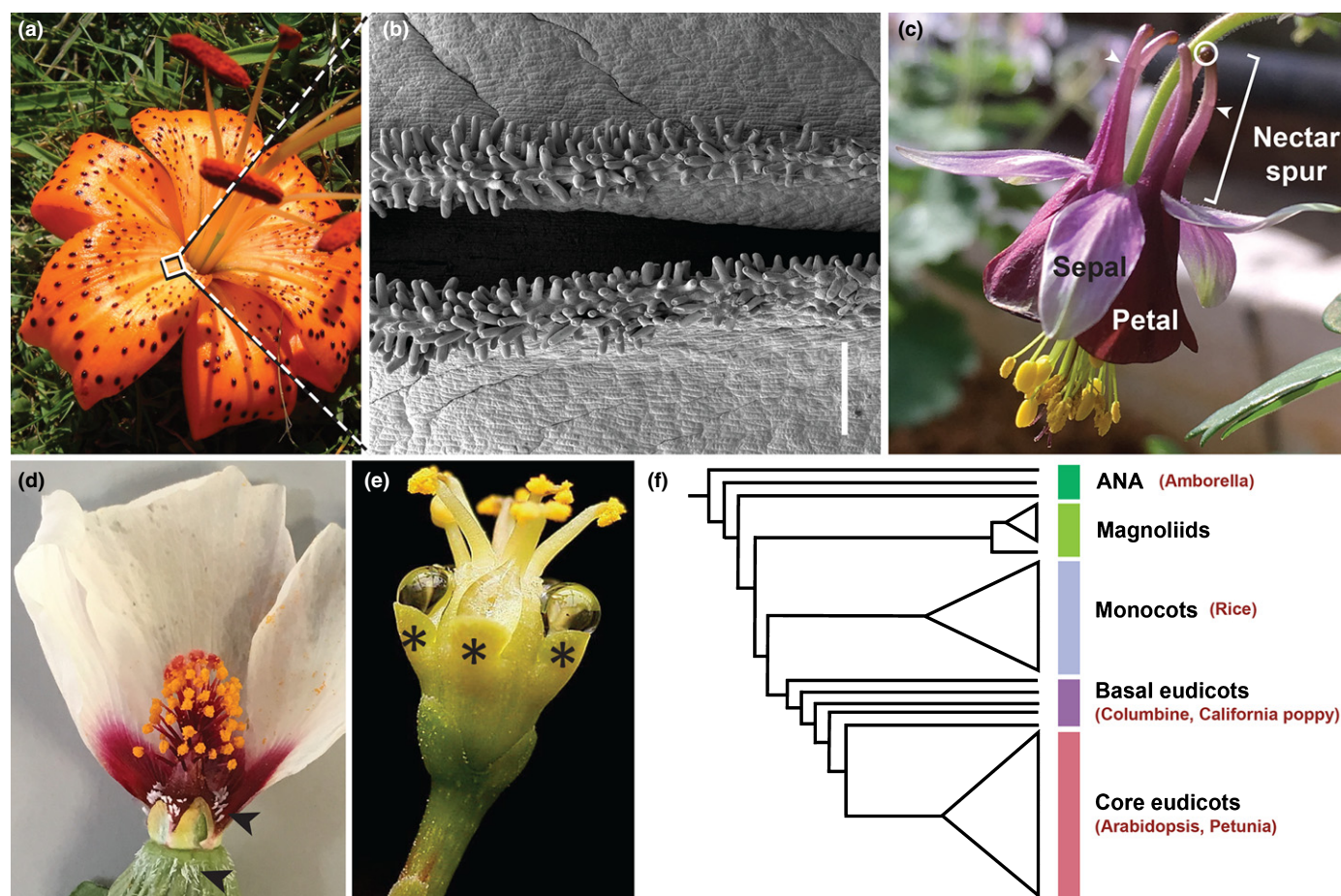
Fig. 1 Diversity of nectaries in flowering plants. (a) The flowers of lilies (*Lilium davidii*) have nectaries on their tepals (boxed region). (b) Scanning electron microscope image reveals that nectaries on *L. davidii* tepals consist of densely packed papillae, arranged on each side of a central slit; bar, 0.5 mm. (c) Flower of *Aquilegia viridiflora* with nectaries (white circle) at the tip of the nectar spur. The spur extremity is filled with nectar (arrowhead) and acts as a receptacle. (d) Floral nectaries (black arrowheads) in *Hibiscus verdcourtii* are secretory white hairs found at the base of the sepals (adaxial surface) and at the base of the ovary. (e) Inflorescence of *Euphorbia enopla* (Frank Vincentz, Wikimedia) showing three extra-floral nectaries (asterisks). (f) Simplified phylogeny of the angiosperms representing the evolutionary relationships between the key groups of extant flowering plants. The position of the main species discussed in the text is also given.

flowers displaying a pistil-phenotype also failed to produce nectar because their nectariferous tissues did not differentiate properly. This establishes *STY*-like genes as regulators of nectary development in basal eudicots and demonstrates that nectar spur formation and nectary development can be uncoupled. But what does this tell us about the evolution of the genetic programme(s) behind nectary formation?

Until the study by Min *et al.*, a single gene was known to control nectary development. Isolated in the late 1990s, *CRABS CLAW* (*CRC*) encodes a YABBY transcription factor essential to induce correct fusion of the carpels and nectariferous gland formation at the base of the Arabidopsis stamens (Bowman & Smyth, 1999). CRC acts as a bifunctional transcription factor: it activates gene expression to promote carpel and nectary development but represses transcription to promote floral meristem (FM) termination (Gross *et al.*, 2018). *CRC* homologues also regulate carpel development in California poppy, a basal eudicot, and expression data from Amborella and rice suggest this role is likely conserved in monocots and in the ANA grade (the earliest diverging lineages of

extant angiosperms) (Yamaguchi *et al.*, 2004; Fourquin *et al.*, 2005; Orashakova *et al.*, 2009). The role of CRC in floral meristem termination is also shared between eudicots and monocots. However, *CRC*-like genes are not expressed in the nectaries of columbine and barrentwort, both basal eudicots (Lee, 2005; Sun *et al.*, 2013). The study by Min *et al.* fills the gap by demonstrating that in those two genera, nectary development is controlled by *STY*-like genes instead.

Who is controlling nectary formation in noneudicots?

So far, expression of *CRC*-like genes in floral nectaries has not been reported outside of the core eudicots. Thus, the nectary-promoting role of *CRC* could be specific to this group. Several species from the ANA or Magnoliids grades produce floral nectaries (Erbar, 2014) and could be used to test this hypothesis by examining whether *CRC*-like genes are expressed in their emerging nectariferous tissues. Alternatively, *CRC* could control the formation of nectaries but only when the nectaries are

associated with reproductive structures. In most core eudicots, including Arabidopsis, nectaries are associated with reproductive organs (Bernardello, 2007) while columbine bears its nectaries on its petals. Recent work shows that the development of stamen- or pistil-associated nectaries in core eudicots is controlled by homeotic MADS-box genes known to specify floral organs identity (Morel *et al.*, 2018). Two sublineages of C-class genes, euAP3 and PLE, act redundantly to activate *CRC* expression and thus induce nectary formation next to the stamen in Arabidopsis or at the base of the carpels in Petunia. Expression of C-class genes is restricted to reproductive organs and absent from petals. This might explain why nectary development in petals occurs via a different petal-specific mechanism, independent from the C-class/*CRC* module. A few species of Ranunculaceae, the family of Columbine, have nectaries associated with reproductive organs: nectaries are found on the ovary of the wood anemone (*Anemone nemorosa*) and nectar is produced by modified sterile stamen in *Pulsatilla turczaninovii*. It would be interesting to test which of *CRC* or *STY* homologues are expressed in those nectaries to see whether the genetic mechanisms behind nectary induction are better inferred by considering the position of a species in the phylogeny, or the position of its nectary within the flower.

One gene to rule them all: identifying the gene regulatory network behind nectary development

It is now clear that disparate nectaries can be induced by the same regulator because *CRC* expression is conserved in morphologically distinct nectaries. *CRC* and *STY* may regulate core programmes inducing nectaries in different parts of the flower but diversity in nectary architecture is likely due to variation among the downstream targets of those master regulators. However, such targets remain to be identified. Both *CRC* and *STY* control organ development in part through regulation of auxin synthesis (Landberg *et al.*, 2013; Yamaguchi *et al.*, 2017). However, nectary development could be auxin-independent: in columbine, the *YUCCA* genes involved in auxin synthesis are not preferentially expressed in tissues where nectaries will form and external auxin application to buds does not induce ectopic nectary formation (Min *et al.*).

Next-generation sequencing techniques and protocols to conduct functional studies in an increasing number of species have boosted our ability to identify key regulators of novel or labile structures. Research in columbine has been pioneering, using natural variation coupled with laboratory-based experimental techniques to explore the genetic regulation of morphological traits impacting on fitness. However, our ability to decipher the mechanics of gene regulatory networks (GRNs) often lags behind our aptitude to single out master regulators controlling those networks. Our capacity to detect *cis*-elements *in silico* and to predict the combined behaviour of those regulatory motifs is still rudimentary. Is there a core GRN for nectary development? How similar are the gene sets targeted by *CRC* and *STY* to control style vs nectary development? How different are the GRNs orchestrating the development of disparate nectaries? These are only a few

examples of outstanding questions in nectary research. Nectaries play a crucial role in plant–pollinator interactions and the evolutionary success of angiosperms. Two hundred years later, Senebier's strong desire for piercing their secrets perfectly resonates with upcoming efforts to decipher the GRNs controlling the development of these remarkable organs.

ORCID

Edwige Moyroud [iD] https://orcid.org/0000-0001-7908-3205

Edwige Moyroud [iD]

The Sainsbury Laboratory, University of Cambridge,
Bateman Street, Cambridge, CB2 1LR, UK
(tel +44 (0)1223 761163; email Edwige.moyroud@slcu.cam.ac.uk)

References

Bernardello G. 2007. *A systematic survey of floral nectaries. Nectaries and nectar.* Dordrecht, the Netherlands: Springer, 19–128.

Bowman JL, Smyth DR. 1999. CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**: 2387–2396.

Erbar C. 2014. Nectar secretion and nectaries in basal angiosperms, magnoliids and non-core eudicots and a comparison with core eudicots. *Plant Diversity and Evolution* **131**: 63–143.

Fourquin C, Vinauger-Douard M, Fogliani B, Dumas C, Scutt CP. 2005. Evidence that *CRABS CLAW* and *TOUSLED* have conserved their roles in carpel development since the ancestor of the extant angiosperms. *Proceedings of the National Academy of Sciences, USA* **102**: 4649–4654.

Gomariz-Fernández A, Sánchez-Gerschon V, Fourquin C, Ferrándiz C. 2017. The role of *SHI/STY/SRS* genes in organ growth and carpel development is conserved in the distant eudicot species *Arabidopsis thaliana* and *Nicotiana benthamiana*. *Frontiers in Plant Science* **8**: 814.

Gross T, Broholm S, Becker A. 2018. CRABS CLAW acts as a bifunctional transcription factor in flower development. *Frontiers in Plant Science* **9**: 1–13.

Landberg K, Pederson ERA, Viaene T, Bozorg B, Friml J, Jonsson H, Thelander M, Sundberg E. 2013. The moss *Physcomitrella patens* reproductive organ development is highly organized, affected by the two *SHI/STY* genes and by the level of active auxin in the *SHI/STY* expression domain. *Plant Physiology* **162**: 1406–1419.

Lee J-Y. 2005. Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* **132**: 5021–5032.

Min Y, Bunn JI, Kramer EM. 2019. Homologs of the STYLISH gene family control nectary development in *Aquilegia*. *New Phytologist* **221**: 1090–1100.

Morel P, Heijmans K, Ament K, Chopy M, Trehin C, Chambrier P, Rodrigues Bento S, Bimbo A, Vandenbussche M. 2018. The floral C-lineage genes trigger nectary development in Petunia and Arabidopsis. *Plant Cell* **30**: 2020–2037.

Orashakova S, Lange M, Lange S, Wege S, Becker A. 2009. The *CRABS CLAW* ortholog from California poppy (*Eschscholzia californica*, Papaveraceae), *EcCRC*, is involved in floral meristem termination, gynoecium differentiation and ovule initiation. *Plant Journal* **58**: 682–693.

Senebier J. 1800. *Physiologie végétale.* Geneva, Switzerland: J.J. Paschoud, 267–283.

Sun W, Huang W, Li Z, Lv H, Huang H, Wang Y. 2013. Characterization of a CRABS CLAW gene in basal eudicot species *Epimedium sagittatum* (Berberidaceae). *International Journal of Molecular Sciences* **14**: 1119–1131.

Yamaguchi N, Huang J, Xu Y, Tanoi K, Ito T. 2017. Fine-tuning of auxin homeostasis governs the transition from floral stem cell maintenance to gynoecium formation. *Nature Communications* **8**: 1125.

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka M, Nagato Y, Hirano HY. 2004. The *YABBY* gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa. Plant Cell* **16**: 500–509.

Yant L, Collani S, Puzey J, Levy C, Kramer EM. 2015. Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20142778.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**