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A model system for comparative research: *Petunia*

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Research today aims to analyse the development of plant processes over evolutionary time. To obtain a representative view, a range of plant species covering at least the crucial nodes in phylogeny must be selected for an in depth analysis. Here we present *Petunia* as one of the available systems: as a representative of the Solanaceae it has the advantages of good culture conditions and the availability of a range of materials, techniques and strategies that can be used to research an interesting and diverse set of questions.

Why an array of model systems?

Plants have evolved to adapt to environmental cues and to conquer and establish new ecological niches. Changes in gene functions and their controlling networks over evolutionary time have enabled the development of innovative structures, such as the reproductive organs in gymnosperms and angiosperms and the many different plant–plant, plant–animal and plant–microbe interaction strategies. Such changes in genetic make-up have ultimately led to the development of the ~250 000 extant plant species.

Whereas systems biology approaches focus on a single species to obtain ultimately a detailed and integrated view of the function of all genes within a single organism, comparative biology has a broader view on (dis)similarities in specific developmental and functional pathways across a variety of plant species [1]. Evolutionary developmental biology, or evo-devo, is a combination of systems biology and comparative biology [2].

One of the challenges ahead for plant scientists is to analyse and to understand the level of diversification that has allowed for the astonishing degree of diversity among the members of the plant kingdom. To understand these developmental differences between species, we must compare gene function development for a range of species covering the evolutionary diversity of all species. It is clear that the scope of plant research needs to be broadened; we could begin with identifying a well-spread set of taxa that would enable us to further unravel the crucial nodes of evolutionary events that have shaped the diversity we see today in plant morphology and in the modes of reproduction and survival.

The most advanced model plant species is *Arabidopsis* but, in spite of its success, it cannot represent all extant species [1], if only for the reason that it is not representative for 'all' plant processes and interaction strategies. Moreover, analysis above the ecotype level is hampered by the difficulty in obtaining fertile progenies from crosses between *Arabidopsis thaliana* and related species [3]; for *Petunia*, this is fairly easy. In addition, *Petunia* occupies a more diverse range than *Arabidopsis*, considering the diversity in forms and ecological niches.

In June 1980, the interim steering committee of the Plant Molecular Biology Association published its first PMB newsletter. In the foreword, *Petunia* and *Lycopersicon* were mentioned as outstanding model systems (but with the remark that 'The main objection to *Petunia* is that it will never be an important food source.'). Among other reasons, 'the availability of true haploids, its easy tissue culture and the quality of leaf tissue for biochemical studies and macromolecule purification' were mentioned, aspects that remain important to this day. The second issue, which featured *Petunia* and *Lycopersicon* as model systems on its cover, was filled almost entirely with information on *Petunia* and *Lycopersicon* model systems. Nevertheless, in the first issue, *Arabidopsis* was also recommended, among others, as a good alternative model system, which was a fairly accurate prediction.

Here we propose *Petunia* as one of the available comparative eudicot systems. We will detail its historical setting, the major technological possibilities of using *Petunia* as a model system and the main areas of current research. We will of course have to balance the use of *Petunia* with models for other groups, for example, rice for the grasses, poplar and *Eucalyptus* for trees and *Medicago* and *Lotus* for the legumes.

Petunia: history and research

The genus *Petunia*, established by Jussieu in 1803, comprises ~30 (sub)species and belongs to the family of the *Solanaceae*. Its main geographical distribution is from Argentina to Uruguay and in the Southern part of Brazil as well as in the Andean foothills [4–6]. Bailey [7] mentions that *Petunia axillaris* (*Petunia nyctaginiflora*) was first cultivated in 1823, and that *Petunia integrifolia* (*Salpiglossis integrifolia*) first flowered in the Glasgow Botanical Garden (UK) in July 1831 ('from seeds sent the fall before from Buenos Ayres by M_r Tweedie'). *Petunia* is considered to be the first cultivated bedding plant and has remained one of the favorite genera for developing new varieties. It was not until the 1950s that geneticists began to try to predict new colour classes from their genetic and biochemical analyses on *Petunia*; until then research had

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been fairly frugal and practice-driven. Some of the new areas of research included work on mosaic flower colour variations [8], flavonoid synthesis [9], floral development [10–12], the claim of naked DNA transformation [13], male sterility [14], self-incompatibility [15,16], tissue culture and regeneration [17]. An exhaustive and still valuable overview of research topics and publications up to 1980 is presented in Kenneth Sink's 1984 monograph '*Petunia*' [18]. In the early 1980s, there were only a few laboratories that focused on *Petunia* as a model system, mainly the Genetics Institute at the University of Amsterdam (The Netherlands) and a group at the INRA (Dijon, France). These two groups started to exchange materials and data in 1978, which led to a merging of information on genetic maps and of mutant collections (including trisomics for each of the seven chromosome pairs). However, despite the passage of time, the genetic map of *Petunia* has remained enigmatically small, probably because of the hybrid nature of the genome of most cultivars [19].

One of the main outcomes of the collaboration between the two groups was the genetic and biochemical description of ~30 structural and regulatory genes involved in flavonoid synthesis [20,21]. In the early 1980s, the classical description of endogenous transposable elements also took shape. The Genetics Institute (founded by Hugo de Vries) at the University of Amsterdam closed down in the mid-1980s and research at the INRA was terminated a few years later, events that nearly ended the existence of the genetic materials.

Around that same time, a new group was set up at the Free University of Amsterdam that ventured into the field of plant molecular biology with *Petunia* as the model system. Over the years the group has contributed to the cloning and characterization of many flavonoid genes (e.g. Refs [22–24]), as well as to the discovery of, for example, antisense and cosuppression [25–28], and the isolation [29] and use [30] of endogenous transposable element systems. Although not a widely recognized model system of choice, *Petunia* has been useful, for example, for developing transformation methods [31], to map transgenes genetically as stable inherited units [32,33], to map transgenes by *in situ* hybridization [34], or to change flower colour using transgenes [35].

From the late 1980s onwards, *Petunia* research proliferated further with progressively more work on various aspects of plant and flower development. Nowadays there are 15–20 groups worldwide that champion *Petunia* as their main model plant system. Parallel to the better known work in *Antirrhinum* and *Arabidopsis*, work on floral development began to take shape; initially this work was undertaken by Arjen van Tunen's research group, and later by Gerco Angenent's research group, at the Plant Research Institute (Wageningen, The Netherlands). Many contributions confirmed, but also expanded or challenged the general conclusions put forward by the *Arabidopsis* and *Antirrhinum* research groups, underlining the value of comparative research by presenting intriguing differences that are more then just ornamental variations on a theme (e.g. [36]). Figure 1 shows a selection of classical and more-recently identified *Petunia* floral developmental mutants.

Some floral development mutants were described a surprisingly long time ago. From 1890 there is a description by Carman (quoted in Ref. [7]) that might have been of a full *leafy* type – 'rosettes of green leaves without the rudiments of calyx, corolla, stamens or pistils'. Joseph Harrison in 1838 (quoted in Ref. [7]) described forms that exhibit 'grass-green borders on a red body', which are reminiscent of the typical *fbp2* mutant greenish corollas [37], and Albert Levan [11] describes a wonderful mutant in which the ovules are replaced by leaf-like structures that eventually break through the carpel wall, reminiscent of the *fbp2 fbp5* double mutant (Figure 1c) as described in [37].

Petunia: present research topics

Today, *Petunia* research has expanded into many different areas, covering comparative as well as unique questions. A brief overview of active research areas is presented in Table 1, many of which exhibit more general implications beyond the model system. Four of these will be highlighted to some extent here; research with *Petunia* in the field of meristem development will be discussed in more detail by Gerco Angenent and colleagues in a Review article [38] and Ronald Koes and colleagues [39] will discuss flavonoid synthesis in more detail in a Review article in this issue of *Trends in Plant Science*.

Retroelement activity

Petunia harbours one retroelement of particular interest, the petunia vein clearing virus (PVCV), which combines features of both viral and non-viral retroelements [40]. Amplification of episomal PVCV induces typical vein clearing symptoms. Integration of PVCV sequences has been preserved in the pericentromeric region of *Petunia* chromosomes and it probably does not compromise expression of important plant genes because its own expression is repressed by its heterochromatin position. Normally integrated PVCV sequences are subject to transcriptional silencing (DNA-methylation). However, various stresses can induce transcription of PVCV

Table 1. Overview of active research areas with some key references

Research areas	Refs
Taxonomy	[4,6,55]
Flavonoid synthesis	[20,21,39]
Meristem activity	[38,56]
Floral development	[36,49,57]
Genetic maps	[19,33]
Transposons	[29,54,58]
Transposontagnology	[30,37,59]
Forward genetics	[60]
Epigenetics	[61–64]
Volatiles	[65–67]
Pollination syndromes	[54]
Retroelement activity	[40,68]
Selfincompatibility	[69,70]
Male sterility	[41]
Expansins	[43]
Transformation of plastids	[71]
Senescence	[72,73]

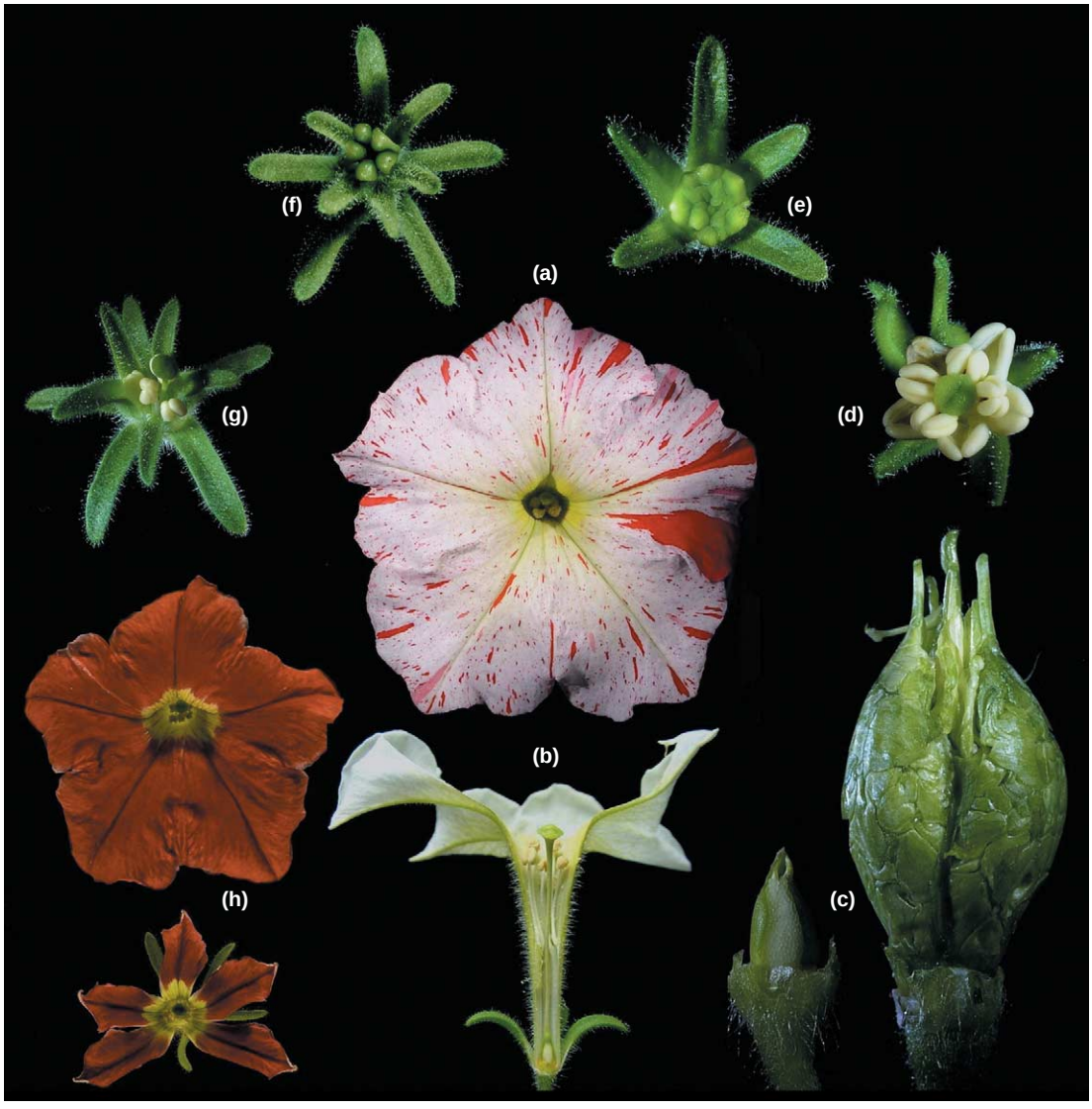

Figure 1. Examples of some *Petunia* floral developmental mutants and standard wild-type lines. Note that not all images are to scale. **(a)** *Petunia hybrida* W138 high copy number *dTph1* transposon variety used for forward and reverse genetics. **(b)** *Petunia hybrida* 'Mitchell' variety (two sepals have been removed and the limb sliced open to show the inner organization of the flower). **(c)** *fbp5* single mutant ovary showing ovules similar to wild type and *fbp2* single mutants (left); giant ovary of *fbp2 fbp5* double mutant showing densely packed leaf-like organs replacing the ovules (right) [37]. The carpel wall, style and stigma in the *fbp5* mutant and in the *fbp2 fbp5* double mutant have been removed to reveal the inner organs. **(d)** *blind* (*bl*) mutant flower with corolla replaced by antheroids [74]. **(e)** *phglo1 phglo2 bl* triple mutant displaying carpelloid organs in whorls 2, 3 and 4. **(f)** *phglo1 phglo2* double mutant with whorls 1 and 2 consisting of sepals and whorls 3 and 4 consisting of carpels [37,49]. **(g)** *phdef* mutant with petals converted to sepals [75]. **(h)** Wild type (top) and *maewest* flower (bottom) showing defects in petal fusion.

sequences in *Petunia* [40]. Special attention should be paid to the inducibility of endogenous PVCV activity in plant breeding programmes and plant regeneration systems using tissue culture.

Male sterility

Maureen Hanson's group at Cornell University (Ithaca, NY, USA) has cloned a mitochondrial gene that encodes Cytoplasmic Male Sterility (CMS) in *Petunia*. The gene encodes an abnormal protein that disrupts mitochondrial activities. A nuclear gene (the *Restorer fertility* or *Rf* gene) is known to interact with this mutant mitochondrial gene, reducing its expression and thereby restoring normal fertility to male sterile genotypes. The group has identified the *Petunia Rf* gene by cloning candidate genes from map position and demonstrating that such a gene is able to confer fertility to CMS *Petunia* lines [41]. Subsequently, genes highly similar to the *Petunia Rf* have been identified as fertility restorers in *Brassica* and rice [42].

Expansin

Mario Pezzotti's group at the University of Verona (Italy) has shown that modulating the activity of a specific expansin reduces the amount of crystalline cellulose in cell walls and leads to phenotypic changes in petal limbs [43]. Expansins are involved in the disruption of the noncovalent bonds between cellulose microfibrils and cross-linking glycans, thereby promoting wall creep. A transgenic antisense approach was used to modulate the expression of the *PhEXP1* α-expansin gene. A decrease in this activity caused a decrease in petal limb size and a reduction in the epidermal cell area, as well as alterations in cell wall morphology and composition. The diminished cell wall

thickness and the reduction in crystalline cellulose indicate that the activity of *PhEXP1* is associated with cellulose metabolism. The results suggest that expansins play a role in the assembly of the cell wall by affecting either cellulose synthesis or deposition [43].

Floral development

Since the early 1990s, extensive molecular studies of plant floral mutants in a range of species have accumulated evidence for the importance of the MADS-box gene transcription factor family in floral development and plant architecture. It has been proposed that gene duplication events and subsequent functional diversification within the MADS-box gene family have been one of the driving forces creating the morphological diversity within the plant kingdom [44,45]. A broad sampling of MADS-box genes in >100 plant species has indeed revealed the existence of several MADS-box gene subclades that are only present in some taxa or systematically absent in others. Studying the function of these genes in particular might accelerate our understanding of the evolution of flower and plant shape. For example, within the *DEF/AP3* subfamily, a major duplication event occurred, presumably at the base of the core eudicots, leading to the eu*AP3* and *TM6* lineages [46]. This duplication event was accompanied by a frameshift mutation in the C-terminal region of the eu*AP3* lineage genes [47,48]. In *Arabidopsis* and *Antirrhinum*, the crucial role of these newly evolved eu*AP3* genes (*AP3* and *DEF*) in petal and stamen development has been extensively studied. However, the evolutionary significance of this duplication event and the 'ancestral preframeshift' function of the *DEF/AP3* lineage remain largely unknown because it cannot be studied in *Arabidopsis* or *Antirrhinum* because both have probably lost their *TM6* representative. By contrast, in *Petunia*, both an eu*AP3* (*PhDEF*) and a *TM6* (*PhTM6*) copy is present. In a recent study of B-function regulation in *Petunia* [49], it became clear that the *PhTM6* function must be significantly different compared with classical *DEF/AP3* genes, based on expression patterns and genetic interactions. Currently, we are focusing on characterizing *phtm6* mutants and the *FBP9* gene, another *Petunia* MADS-box protein for which no clear orthologue is present in the genome of *Arabidopsis* [50]. An aspect of flower development that cannot be studied in *Arabidopsis* is petal fusion. In a genetic screen, we isolated *maewest* (*maw*) mutants in which petal fusion is distorted (Figure 1h). We have now cloned *MAW* and are analysing the mutant in more detail.

Qualities of the *Petunia* system

Some of the important qualities of the *Petunia* model system are:

- Easy growth habit and relatively short lifecycle of roughly four months from seed to seed.
- Easy asexual propagation from cuttings, callus or protoplasts.
- Easy transformation procedures for a defined set of varieties (including the species *P. axillaris*).
- Large and expanding set of functionally and molecularly well characterized genes involved in, for example, flavonoid synthesis and diverse aspects of plant development.
- Availability of large sets of mutants, mainly caused by insertion of endogenous transposable elements.
- Sophisticated methods for forward and reverse screenings of such mutants.
- Intriguing genome behaviour (genetic linkage maps are among the smallest on record, indicating huge blocks of recombination inhibition, which might be related to the hybrid origin of most cultivars).
- Amenity for cytogenetic analysis.
- Amenity for biochemical analysis because of its large leaves and flowers.
- Growing availability of many molecular tools such as (organ-specific) cDNA libraries, insertion libraries, BAC libraries, growing EST collections and microarrays.
- Dynamic and cooperative research platform.

Large mutant collections are maintained by several groups and many seed companies have widely diverse collections of germplasm (the origin of which is mostly not publicly available). Toshio Ando and co-workers have a huge number of samples collected from defined natural populations.

There are three useful greenhouse varieties: the Mitchell variety [51] (Figure 1b), which is a doubled haploid from a complex hybrid between *P. axillaris* and the cultivar 'Rose of Heaven' that exhibits superior fertility, growth, tissue culture and transformation abilities; the line V26, a bluish purple line that has been used for antisense and cosuppression studies, flavonoid gene isolation and ethyl methane sulfonate mutagenesis; and the line W138 (Figure 1a), which is practically untransformable but is renowned for its active endogenous *dTph1* transposable element system and which has already produced many interesting mutants [37,52,53]. A recombinant inbred line collection of species crossed with W138 lines and a collection of 1250 stabilized insertion lines is also available [54].

A major advantage of *Petunia* is that the system combines so many excellent technical features with a broad range of research possibilities and topics. One of the main areas lacking research at the moment is a genome sequencing project; however, the sequencing of the tomato genome under the International Solanaceae Genomics Project (SOL) (http://sgn.cornell.edu/) might provide sufficient or significant information through (micro)-syntenic relationships or might provoke a comparative sequencing project. Furthermore, *Petunia* as a model excels in areas such as the use of endogenous transposable elements for forward and reverse genetics and the analysis of diverse topics such as branching patterns, volatile production, pollination syndromes and mycorrhiza–plant interactions.

An overview of groups working with *Petunia* can be found at http://www.petuniaplatform.net where keyword descriptions of the research of each group are presented, together with links to their respective websites. The website also details services offered and we will develop

a dynamic annotated bibliography and an on-line monograph. We invite you to go through our website for detailed further information.

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