

Commentary

Plant formins come of age: something special about cross-walls

In this issue of *New Phytologist* (Deeks *et al.*, pp. 529–540), Patrick Hussey and his coworkers report on the very distinct localization of *Arabidopsis thaliana* formins, AtFH4 and AtFH8, to cross-walls of roots, hypocotyl and shoot tissues. This is the first time that plant formins are reported to have such distinct domain-specific subcellular localizations. Here we discuss these pertinent findings from the broader perspective of plant cell polarity, cell wall–cytoskeleton adhesion domains, polar auxin transport, and the emerging unique status of these cross-walls in that they resemble neuronal and immunological synapses (Baluška *et al.*, 2003a,b,c, 2005).

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In 2000, Fatima Cvrčková carried out a bioinformatic approach to search for plant formins; eight putative formin-coding genes were identified (Cvrčková, 2000). It was of great surprise to find that the majority of these plant formins contained potential transmembrane domains which are not present in the yeast and animal formins, and suggests that they are integral membrane proteins. Moreover, some of the plant formins also contained an exposed proline-rich domain which presumably inserts into the cell wall, exhibiting similarity to cell wall extensin. From these bioinformatic analyses, it was predicted that some plant formins might be involved in the direct cell wall–cytoskeleton communication (Cvrčková, 2000; Baluška *et al.*, 2003c). During the last two years, our knowledge of plant formins has literally expanded. Bioinformatics data are now well

advanced (Cvrčková *et al.*, 2004), and there are also first data on both cell biology (Cheung & Wu, 2004; Favery *et al.*, 2004; Ingouff *et al.*, 2005; Yi *et al.*, 2005) and biochemistry (Michelot *et al.*, 2005; Yi *et al.*, 2005). This most recent study by Deeks *et al.* opens new avenues in our understanding of plant-specific formins as they report domain-specific enrichment of AtFH4 and AtFH8 at cross-walls of diverse plant organs. This raises the intriguing question as to what is so specific about these cross-walls.

Cross-walls: from auxin transport to vesicle recycling

Cross-walls of longitudinal cells have long been suspected to have special signaling properties, simply because it has been known for many years that developmental signals and cues are spread primarily along the longitudinal axis of organs such as roots and stems (Baluška *et al.*, 2003a). Moreover, the cross-walls are known to harbour numerous plasmodesmata which interconnect adjacent cells of cell files into syncytium-like supercells (Baluška *et al.*, 2003a,c). Recently, the interest into these subcellular domains increased further as a result of advances made concerning proteins that assist in the polar transport of auxin. Because this plant hormone is transported preferentially along cell files, the cross-walls inevitably represent domains which are transporting auxin from cell to cell (Baluška *et al.*, 2003b, 2005).

For unknown reasons, auxin is not transported across plasmodesmata even though its small size should guarantee a free passage. In light of this, a theoretical model was proposed and followed which considers plasma membrane transporters (putative influx and efflux carriers) which should drive the polar transport of auxin across cross-walls (reviewed by Friml & Palme, 2002; Friml & Wiśniewska, 2005). This so-called chemiosmotic model is based on the chemical properties of auxin and acidic pH values found in cell walls vs basic pH values of the cytoplasm. In accordance with this model, putative auxin transporters are localized in a polar fashion (reviewed by Friml & Wiśniewska, 2005). Further new data are rather at variance with this model. For instance, although the early concept of polar auxin transport did not consider vesicle trafficking at all, this process slowly penetrated all the papers dealing with the auxin transport because putative efflux carriers turned out to perform rapid recycling between the plasma membrane and putative plant endosomes (Friml & Wiśniewska, 2005). The chemiosmotic model has difficulties to explain why plasma membrane transporters undergo such a rapid recycling rate. Nevertheless, one can easily explain this point by invoking impacts of

developmental cues and signaling cascades on the flow of auxin via rapidly reshifting polar subcellular localizations of these putative auxin transporters.

However, there are more serious weak points of this model which claim the plasma membrane transporter should drive transcellular transport of auxin. First of all, despite the suggestive polar localization of putative transporters (including PIN1, PIN2, PIN3, PIN4 and AUX1) to the cross-walls (reviewed by Friml & Wiśniewska, 2005), all attempts to prove the plasma membrane transporter nature of these proteins has failed until now. Hence, the consensus of opinion has been slowly shifting to consider the carrier nature of these proteins as transport facilitators or regulators. Secondly, brefeldin A (BFA; a potent inhibitor of vesicular secretion in plants, as in other eukaryotic cells) blocks polar auxin transport within a few minutes of application (Delbarre *et al.*, 1998; Paciorek *et al.*, 2005). Importantly, BFA not only exerts rapid inhibition of the auxin efflux but also causes the complete block of this process, whereas auxin influx is not affected (Delbarre *et al.*, 1998). Because BFA blocks exocytosis while it stimulates endocytosis (Wang *et al.*, 2005), these rapid effects of BFA on the polar auxin transport correspond well to the 'neurotransmitter' nature of auxin, being secreted out of exporting cells and perhaps taken up via endocytosis by a receiver cells (Baluška *et al.*, 2003b; Friml & Wiśniewska, 2005). Finally, inhibitors of the polar auxin transport, irrespective of their chemical nature, turned out to act as inhibitors of endocytosis (Geldner *et al.*, 2001, 2003). From the chemiosmotic model perspective, it is a mystery why inhibitors of endocytosis should block the polar transport of auxin, whereas the alternative 'neurotransmitter' model (Friml & Palme, 2002; Baluška *et al.*, 2003b; Friml & Wiśniewska, 2005) can easily explain this conundrum.

Although all PINs as well as AUX1 are known to accomplish vesicular recycling at the cross-walls, and although they get trapped into the endocytic BFA-induced compartments in BFA exposed cells, this happens only after some 10–15 min, whereas the full size of BFA compartments is achieved only after 120 min (Geldner *et al.*, 2001, 2003). However, BFA inhibits polar transport of auxin immediately after exposure, when most of the carriers are still localized to the plasma membrane (see fig. 2o in Paciorek *et al.*, 2005). In addition, even after 90 min of BFA treatment, when BFA-induced compartments have reached large size, there is still a considerable portion of auxin carriers localized to the plasma membrane at cross-walls (Paciorek *et al.*, 2005). Again, this is at variance with the chemiosmotic model but corresponds with the 'neurotransmitter' model of polar auxin transport.

The only known process which is blocked within a few minutes of BFA exposure is vesicular secretion, irrespective of whether it is the constitutive Golgi-apparatus-based secretion or the endocytosis-based and vesicle-recycling-driven regulated secretion (Šamaj *et al.*, 2005). Therefore, the most plausible explanation of the very rapid blockage of auxin

transport via BFA is that auxin is secreted via recycling-based regulated secretion. In support of this latter notion, we have recently localized auxin into vesicular structures as well as within endocytic BFA-induced compartments (our own unpublished data). Moreover, depolymerization of F-actin, which prevents endocytosis of auxin carriers (Geldner *et al.*, 2001, 2003), and thus maintains them at the plasma membrane, inhibits polar auxin transport too (Sun *et al.*, 2004). This latter finding is again at variance with the chemiosmotic model. Importantly, the F-actin-dependence of polar auxin transport implicates that F-actin nucleators will be critical for our mechanistic understanding of processes driving polar auxin transport across cellular boundaries.

Cross-walls: actin-, myosin VIII- and formin-enriched domains specialized for endocytosis, rapid vesicle recycling and signaling

Cross-walls are known to be actin-enriched domains (Baluška *et al.*, 2003a). This is rather surprising because cross-walls are nongrowing domains in postmitotic root cells. Most eukaryotic cells, with the exception of neuronal synapses, assemble dense F-actin meshworks typically at growing domains (Baluška *et al.*, 2003a). Recently, however, it is becoming clear that extensive endocytosis and vesicle recycling (Šamaj *et al.*, 2005) is going on under nongrowing cross-walls, which balances exocytosis to such an extent that there is no net growth of cell periphery at these highly specialized subcellular domains (Baluška *et al.*, 2003a,b,c). Thus, cross-walls resemble the neuronal synapses (Baluška *et al.*, 2003b) and the imperative question that emerges concerns what molecules act as actin nucleators at the synaptic cross-walls.

The first obvious candidate was the ARP2/3 complex; indeed, knocking out this complex results in disassembly of cross-walls in epidermal cells (Basu *et al.*, 2005; Mathur, 2005). However, cross-walls of nonepidermal cells remained intact in cells devoid of the ARP2/3 complex (reviewed by Mathur, 2005) and the overall phenotype of these mutants are mild, suggesting the existence of another powerful F-actin nucleator. Indeed, recent studies confirmed that plant formins are potent F-actin nucleators (Michelot *et al.*, 2005; Yi *et al.*, 2005). Therefore, the recent finding by Deeks *et al.*, that AtFH4 and AtFH8 are localized to the cross-walls of roots, hypocotyl and shoot tissues, is relevant for our understanding of the nongrowing but extremely dynamic actin-based cross-walls. In addition to actin, cross-walls are enriched also with myosins of the class VIII type, and both actin and myosin VIII are known to be important for endocytosis (reviewed by Šamaj *et al.*, 2005). Moreover, profilin was also localized to cross-walls (Baluška *et al.*, 2001a) and, interestingly in this respect, AtFH4 binds to profilin and affects actin polymerization (Deeks *et al.*). Besides the putative auxin transporters, cell wall pectins are also internalized via the same recycling pathways and become trapped within

the BFA compartments under the exposure of root apices to BFA (Šamaj *et al.*, 2005). Pectins are well known to act as adhesive agents of plant cells (Lord & Mollet, 2002), and their recycling at the cell-cell adhesive cross-walls suggests that their function is tightly controlled via these recycling processes, which themselves are targets of developmental cues and signaling cascades. In fact, cross-walls in root apices are also enriched with plant Rho GTPases known as ROPs (Molendijk *et al.*, 2001) and MAP kinases (J. Šamaj, University of Bonn, pers. comm.).

Cross-walls as actin- and pectin-based adhesion domains: do formins and myosins of the class VIII act as elusive adhesive molecules of plant cells?

Plants lack integrins and it still remains a mystery as to which molecules act as the linkers between cell wall components and the cytoskeleton. Recently, we surveyed all candidates and proposed that myosins of the class VIII and formins represent the best candidates for this role (Baluška *et al.*, 2003c). Myosin VIII is enriched at subcellular cell periphery sites involved in callose synthesis and it is possible that it binds directly to one of the callose synthase subunits. This would interlink the cell wall with cytoskeleton via callosic cell periphery domains at plasmodesmata and pit-fields. On the other hand, plant formins of the group I are equipped with an extensin-like domain which is predicted to be inserted into the cell wall (Cvrčková, 2000; Cvrčková *et al.*,

2004). Again, this would provide plant cells with a direct linkage between the cell wall and cytoskeleton.

These two types of cell wall–cytoskeleton linkages would satisfy the plant-specific demands for a very dynamic cell periphery because plant cells often suffer from osmotic stress and respond with very rapid retraction of their plasma membrane/protoplast from the cell wall (Baluška *et al.*, 2003c). Interestingly, this is associated with very rapid callose synthesis, especially to pit-fields at cross-walls, and recruitment of myosin VIII to these sites of callose synthesis (Wojtaszek *et al.*, 2005). It would be interesting to test if formins, too, are recruited to these sites of enhanced adhesion sites.

Formins as synaptic proteins of plants?

More than 100 years ago, Bohumil Němec described in great detail very prominent longitudinal F-actin cables interacting at cross-walls, using the classical cytological methodology (Fig. 1a; Němec, 1901). Today, actin antibodies reveal two different types of F-actin arrays assembled at cross-walls. The first one is the very dense submembraneous meshwork (Fig. 1b) which is involved, together with myosin VIII, in the endocytosis and vesicle recycling. The second one is composed of distinct cables which traverse the cell longitudinally, interconnecting the opposite cross-walls and typically contacting the nuclear surface (Fig. 1b). Both the meshworks as well as longitudinal cables are essential for cell-to-cell communication and are sensitive to BFA (Fig. 1c). Formins are predicted to organize both these F-actin arrays:

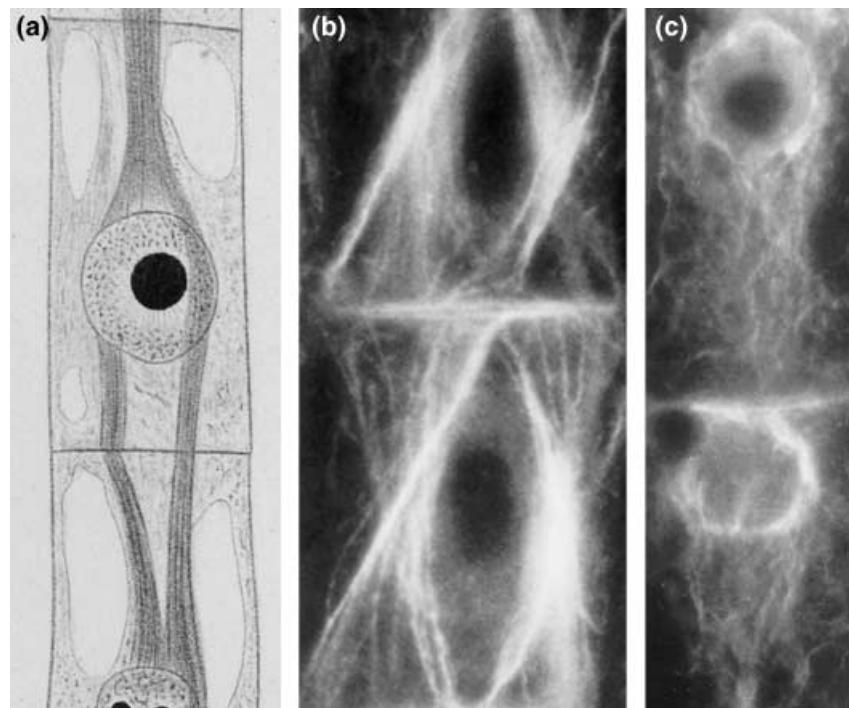


Fig. 1 Actin cytoskeleton in root cells entering the elongation region. (a) Drawing taken from Němec (1901) showing longitudinal bundles traversing the cell, contacting the centrally located nucleus and interacting with the cross-wall. (b) Control root cells of the transition zone showing dense F-actin meshworks at cross-wall and prominent F-actin bundles ensheathing the central nucleus and contacting the cross-wall. (Reproduced from Baluška *et al.* (2001b) with kind permission of Springer Science and Business Media.) (c) After 120 min of BFA (10^{-4} M) treatment, both the cross-wall associated F-actin meshworks and thick cables disassemble and transform into less organized loose arrays. Note that the nuclei were shifted basally (up against the gravity vector), suggesting that their central position is related to the actin cytoskeleton assembly and/or BFA-sensitive vesicle recycling.

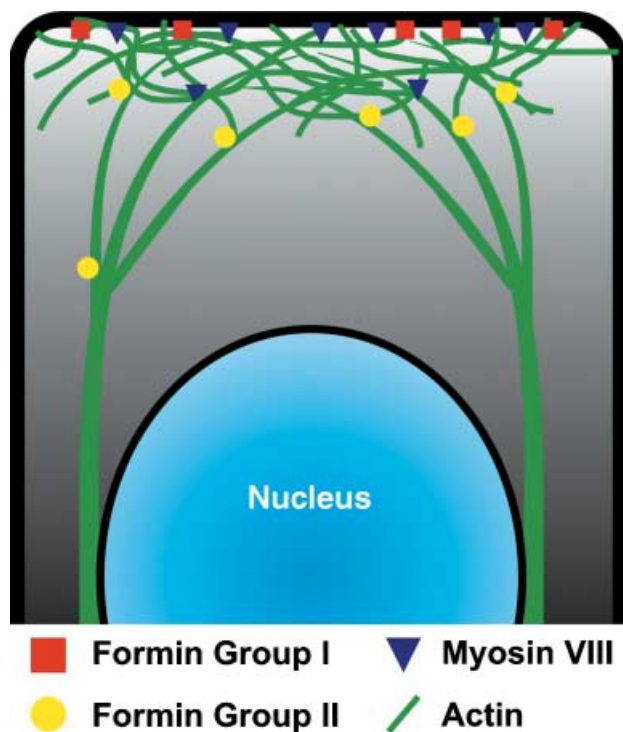


Fig. 2 Schematic presentation of synaptic cross-walls showing the predicted (and, for the group I formins, the confirmed) subcellular localization.

the group I formins which associate with cross-walls (Deeks *et al.*) may be relevant for the dense meshworks, whereas the group 2 formins can be expected to be important for assembly of thick cables (Fig. 2). The dynamic meshworks drive the endocytic recycling related to chemical neuronal synapses as well as immunological synapses. On the other hand, bundles interconnect the opposite cross-walls enriched with plasmodesmata, which might act as electrical neuronal synapses, and hence be involved in the rapid spread of signals, as proposed by Němec. Future studies will unveil how formins nucleate diverse arrays of F-actin at plant synapses in response to developmental cues and signaling cascades which ultimately impinge on the auxin transporting machinery that is based on BFA-sensitive vesicle recycling.

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Key words: adhesive molecules, cell–cell communication, cell wall, cytoskeleton, endocytosis, formins, plant synaptic relations, vesicle recycling.

Arsenic metabolism in plants: an inside story

From murder plots to environmental disasters, arsenic (As) has always been in the headlines. As-contaminated soils, sediments and water supplies are major sources of food chain contamination and thereby endanger human health; this is a global problem, but the situation is nowhere worse than in India and Bangladesh, where more than 400 million people are affected by As poisoning in drinking water (Chakraborti *et al.*, 2003). Inorganic species of As, arsenate (AsO_4^{-3} , referred to as As^{V}) and arsenite (AsO_3^{-3} , referred to as As^{III}), are carcinogenic and have been shown to cause cancer of the lung, liver and kidney and to cause skin pigmentation. Plants too are affected by As; it is a nonessential element and, in general, inorganic As species are phytotoxic. However, some plant species such as *Pteris vittata* – a Chinese brake fern – have been shown to accumulate high levels of As and hence offer a viable opportunity for the remediation of contaminated environments (Ma *et al.*, 2001). To capitalize on these unique remediation capabilities of plants, it is imperative that we understand the mechanisms by which As tolerance and

hyperaccumulation is achieved. We are just at the beginnings of unravelling this story, but a study by Raab *et al.* in this issue (pp. 551–558) provides an important step forward in understanding the mechanistic details of As detoxification in plants.

'It is believed that plants trap arsenite below ground to prevent access to above-ground reproductive tissues in order to prevent possible mutagenic consequences'

Mechanism of arsenic uptake and detoxification in plants

Although largely unknown in plants, the mechanisms of As detoxification have been well characterized in bacteria and yeast, which commonly achieve tolerance to As by the reduction of As^{V} to As^{III} by arsenate reductase enzymes, and then the exclusion of toxic oxyanions As^{III} from the cell by inducible and selective transporters (Mukhopadhyay *et al.*, 2002; Rosen, 2002). To date, no functional orthologs of these microbial arsenate reductases and As^{III} transporters have been identified in plants and thus there is currently no evidence to suggest that plants use these same mechanisms. Recently, however, in addition to the natural As-hyperaccumulating Chinese brake fern, several plants with increased As tolerance have been identified (Meharg & Hartley-Whitaker, 2002). Although the molecular mechanisms of As detoxification and tolerance remain to be fully determined, it has been shown that plants detoxify As by reducing As^{V} to As^{III} (Pickering *et al.*, 2000; Dhankher *et al.*, 2002), which is subsequently detoxified via forming complexes with thiol-reactive peptides such as γ -glutamylcysteine (γ -EC), glutathione (GSH) and phytochelatins (PCs) (Pickering *et al.*, 2000; Hartley-Whitaker *et al.*, 2001; Dhankher *et al.*, 2002; Li *et al.*, 2004). These As^{III} -thiol complexes are then suggested to be sequestered into vacuoles by glutathione-conjugating pumps (GCPs) (Dhankher *et al.*, 2002; Wang *et al.*, 2002), although direct evidence of this remains to be proven. In this issue, Raab *et al.* add new insights into the mechanistic details of As detoxification in plants; they report an extensive study of time-dependent formation of various arsenite–PC complexes in the roots, stems and leaves of sunflower (*Helianthus annuus*) in response to As exposure.

Multiple arsenic species: multiple tolerance mechanisms?

In As-nontolerant sunflower (*H. annuus*), Raab *et al.* use a sophisticated technique to show the formation of 14 different

As species, including some that form complexes with arsenite ($\text{As}^{\text{III}}\text{-PC}_3$, $\text{GS-As}^{\text{III}}\text{-PC}_2$, $\text{As}^{\text{III}}\text{-(PC}_2)_2$) and newly identified monomethylarsonic- PC_2 ($\text{MA}^{\text{III}}\text{-PC}_2$) in response to As exposure. Previously, Hartley-Whitaker *et al.* (2001) demonstrated that the As-tolerant nonhyperaccumulator *Holcus lanatus* contained PC_2 in an As-tolerant clone and PC_3 in nontolerant clone as dominant species. As tolerance in *H. lanatus* was found to result via suppression of the high-affinity phosphate/arsenate uptake system (Meharg & Macnair, 1992), which decreases the arsenate influx into plant roots (Hartley-Whitaker *et al.*, 2001). Similarly, in the As hyperaccumulator *P. vittata*, PC_2 and PC_3 are reported as the major phytochelatin species induced in response to As exposure (Zhao *et al.*, 2003). *P. vittata* differs from *H. lanatus* and sunflower, in that a large amount of As is translocated and stored in above-ground tissues and less As is retained in the rhizome (Ma *et al.*, 2001; Wang *et al.*, 2002). Furthermore, most of the As translocated in the frond is in the form of unbound As^{III} . Clearly, there is much to unravel here.

Raab *et al.* reported two important findings. First, the formation of monomethyl As species complexed with PC_2 ($\text{MA}^{\text{III}}\text{-PC}_2$) in sunflower. Metabolism of As^{V} to organic As species such as dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) has also been observed in phytoplankton, macrophages (Phillips, 1990) and at low concentration in some terrestrial plant species (Koch *et al.*, 2000). These methylated forms of As are then metabolized to organophospholipids and arsenosugars such as arsenobetaine (Phillips, 1990). These findings, taken together with the new results of Raab *et al.*, indicate the existence of an alternative mechanism of As detoxification in plants that warrant further investigation. Furthermore, Raab *et al.* revealed several As complexes, which are yet to be identified and hence point towards further experimental work in order to elucidate the biological complexity of As detoxification.

Arsenic: from roots to shoots

The second important finding was the presence of unbound As^{V} and As^{III} and the absence of As-PC complexes in the sap harvested from sunflower plants exposed to arsenate. Similar results were reported in Indian mustard (*Brassica juncea*) by Pickering *et al.* (2000), in which they identified unbound As^{V} and As^{III} species in xylem sap. Raab *et al.* found only PC_2 and GSH as the main thiol species in sunflower sap, and PC_3 was absent. They concluded that PC_2 and GSH can undergo long-distance transport in *H. annuus*, which is in accordance with earlier results from transgenic *Arabidopsis thaliana* lines overexpressing the wheat phytochelatin synthase gene, *TaPCS1* (Gong *et al.*, 2003). This led Raab *et al.* to postulate that As^{V} and As^{III} are the main species of As that are translocated from roots to shoot tissues via the xylem and not the $\text{As}^{\text{III}}\text{-PC}$ complexes. However, it should be

considered that the results by Gong *et al.* (2003) indicate that, in transgenic *Arabidopsis*, long-distance Cd^{2+} transport is PC-dependent, and therefore further work is required to substantiate these findings.

Previous studies in *B. juncea* and *Arabidopsis* (Pickering *et al.*, 2000; Dhankher *et al.*, 2002), together with the study of Raab *et al.* in sunflower, showed that a major fraction of the As^{V} taken up by plants was retained in roots and that As^{V} was further reduced to As^{III} by endogenous arsenate reductase. Furthermore, most of the As^{V} in roots was in the form of arsenite-thiol complexes. This suggests that plants have an adaptive mechanism, and it is believed that plants trap arsenite below ground in order to prevent access to above-ground reproductive tissues to prevent possible mutagenic consequences. Although several studies suggested the reduction of As^{V} to As^{III} by endogenous arsenate reductases inside plant cells (Pickering *et al.*, 2000; Dhankher *et al.*, 2002), until very recently no enzymes had been identified from higher plants. We have now identified a gene encoding a putative endogenous arsenate reductase from *A. thaliana* that reduces As^{V} to As^{III} in plants (O. P. Dhankher & R. B. Meagher, unpublished). The inactivation of this putative arsenate reductase by RNA interference (RNAi) in *Arabidopsis* enhanced the long distance translocation of As from roots to shoot tissues and thus caused a 10- to 15-fold increase in accumulation of As in above-ground tissues. Duan *et al.* (2005) also recently reported the presence of an arsenate reductase activity from a root extract of *P. vittata* that reduces As^{V} to As^{III} in *in vitro* assays.

In plants, the mechanism of As^{III} uptake and further translocation of As^{III} from roots to shoots also remains to be elucidated. There is strong evidence that AsO_4^{-3} and phosphate (PO_4^{-3}) are taken up by the same transporters in plant roots (Meharg & Macnair, 1992; Wang *et al.*, 2002), but it is not known how and what form of As is translocated from roots to shoots. Compared to As^{V} and PO_4^{-3} , whose chemical properties are very similar, As^{III} is quite different. Only the translocation of As^{V} would therefore be expected to occur via the PO_4^{-3} translocation pathway. Both species of As^{V} and As^{III} were found in xylem sap from stems of *B. juncea* (Pickering *et al.*, 2000) and sunflower (Raab *et al.*); however, it is not known whether both species were actually loaded in the xylem sap or occurred as a result of the reduction and oxidation of As species during translocation in the xylem sap. In another study, Quaghebeur & Rengel (2004) suggested that As^{III} is the main As species translocated from roots to shoots in *A. thaliana*. These preliminary studies suggest that plants may have As^{III} transporters in roots that translocate As^{III} from roots to shoot tissues.

Wider perspectives: phytoremediation

The need to develop efficient strategies for cleaning As-polluted soil and water and also to reduce uptake of As in food

crops to minimize the risk of As contamination through the food chain is clear. Physical remediation methods are highly expensive and not practical at the scales required. Phytoremediation, a plant-based technology, however, holds great promise for the purification of contaminated soil and water (Meagher, 2000). For example, the natural As-hyperaccumulating fern *P. vittata* can be used to clean contaminated soils. However, the mechanism of As hyperaccumulation is not understood and this fern is restricted in growth to the tropics of the southern hemisphere and may not be highly effective in temperate climatic conditions. The uptake and hyperaccumulation capacity of plants can be significantly enhanced by genetic engineering; however, the progress towards developing such genetics-based strategies has been hindered by a lack of thorough understanding of the basic molecular and biochemical mechanisms of As uptake and detoxification in plants. Previously, we developed transgenic plants by overexpressing two bacterial genes, arsenate reductase (*ArsC*) and γ -glutamylcysteine synthetase (γ -*ECS*), in *Arabidopsis* (Dhankher *et al.*, 2002). These plants were super-resistant to arsenate and accumulated a substantially high amount of As in above-ground tissues. Although the potentials of natural as well as genetically modified As hyperaccumulators have raised hopes of reducing As toxicity of water and soil, attention should be focused on engineering high-biomass, fast-growing nonfood plants for soil remediation and aquatic plants for water remediation.

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Key words: arsenic (As), arsenic–phytochelatin (As–PC) complex, arsenic speciation, phytochelatin, phytoremediation.

Letters

Phenotypic plasticity and a functional vs genetic perspective of plant gender

The adoption of a functional perspective of gender constituted an important milestone in the study of plant sexual systems (Lloyd, 1976, 1977, 1980). This perspective formally recognised that a plant's gender depends both on its own sex allocation (i.e. its investment in male vs female functions) and on the sex allocation of other individuals in the population (Lloyd, 1980). It also paved the way for the analysis of plant sexual systems, and the testing of relevant evolutionary hypotheses, with reference to the phenotypic distribution of sex allocations within populations. This phenotypic, or functional, perspective of plant gender has greatly advanced our understanding of plant sexual strategies, and was the main conceptual framework for the recent review of Delph & Wolf (2005) of gender plasticity in populations with dimorphic sexual systems.

In their section on gynodioecy, where females and hermaphrodites co-occur, Delph & Wolf (2005) re-emphasise the idea that gender of the hermaphroditic class may often be plastic in their gender, and that individuals in this class may, in the extreme, fail to produce any seeds (Darwin, 1877; Burrows, 1960; Webb, 1979). The frequency of females at equilibrium will depend on the average gender of individuals in the hermaphroditic class, irrespective of the plastic nature of gender expression. Thus, if conditions cause individuals in the hermaphroditic class to allocate reproductive resources almost entirely to their male function, then the frequency of females will approach 0.5; in contrast, if (e.g. under more benign conditions) the hermaphrodites disperse more pollen, then the female frequency will fall. Importantly, the equilibrium frequency of females will correspond to the phenotypic gender of the hermaphrodite class, irrespective of how gender is determined developmentally.

Although much of their argument followed the functional perspective, Delph & Wolf (2005) also championed a 'population-genetic perspective' in their section on androdioecy, where males co-occur with hermaphrodites. This view seems to differ from the functional perspective. For instance, Delph & Wolf (2005, p. 126) question whether 'a species can be considered androdioecious' if there is a plastic component to the determination of gender in males. They state that, 'from a population-genetic perspective, it is the fitness and functional gender of the morphs across both gender phases that will determine the persistence of each gender morph, and is the

relevant factor to define the breeding system', and they conclude that a population composed of hermaphrodites and a morph that spends most of its time in the male phase might be regarded as 'essentially androdioecious' (Delph & Wolf, 2005, p. 126).

The view that androdioecy needs to be defined in qualified terms when there is a plastic component to gender has also been expressed elsewhere (e.g. Wolf *et al.*, 1997; Webb, 1999). In the extreme, such qualifications give precedent to a classing of the gender of individuals according to their genotype, rather than simply to their function. In general, however, the grounds for a genetic vs functional assessment of gender are left ambiguous. The functional view provides a robust way of making predictions about equilibrium gender frequencies in sexually dimorphic populations, but models that form the foundation of these predictions tend to have been coined in genetic terms. It is thus pertinent to ask how important the genetic view in these models actually is.

Delph & Wolf (2005, p. 126) state their case clearly: 'A better understanding of the breeding system [where genotypes may be either phenotypically plastic or canalised for gender] would result from a comparison of the proportion of fitness that the two [genetic] morphs (plastic and canalized) gain through male function in the field.' This perspective gives precedent to a classing of individuals in the population according to their genotype. The functional perspective, in contrast, views individuals first and foremost in terms of their phenotypes. The functional perspective does not deny the importance of the genetic architecture of populations for understanding or predicting evolutionary trajectories (see the Discussion section below), but it begins by classing individuals in terms of their relative contributions through male vs female functions.

In this Letter, I ask whether established evolutionary theory on plant sexual-system evolution applies better to the phenotypic categories of male, female and hermaphrodite, or to the genotypic morphs of a population-genetics perspective. For the sake of illustration and brevity, I focus here on the example of androdioecy, but the basic argument should apply to a population with any mix of genders. I reframe an established model for the evolution and maintenance of androdioecy by explicitly including a plastic component to the determination of maleness. I thus recognise the genotypic morphs referred to by Delph & Wolf (2005), and I consider whether a potentially plastic behaviour in one of these morphs alters the predictions of the more general model.

Model

Assume that a large population comprises individuals that express one of two genetically determined gender strategies,

which we shall call genotypes. Individuals of genotype 1 are always hermaphrodites, whereas individuals of genotype 2 are hermaphrodite with probability q and male with probability $(1 - q)$. Let f_1 and f_2 be the frequencies of genotypes 1 and 2, respectively, with $f_1 + f_2 = 1$. We assume that all hermaphroditic phenotypes allocate proportions a and $(1 - a)$ of their reproductive resources to their male and female functions, respectively. If we assume a linear increase in male fitness with allocation to the male function and that all pollen grains dispersed by individuals in the population compete on an equal basis to fertilise ovules, then males produce and disperse $r = 1/a$ times more pollen than do hermaphrodites. Hermaphrodites self-fertilise a proportion s of their ovules, and the viability of selfed offspring is $1 - \delta$ times that of outcrossed offspring (i.e. δ denotes the inbreeding depression suffered by selfed progeny). If all ovules are fertilised, then we can write the fitness of genotypes 1 and 2 as

$$w_1 = g(1 - a)(s - 2s\delta + 1) + \frac{pa}{P}G$$

$$w_2 = qw_1 + (1 - q)\frac{p}{P}G, \quad \text{Eqn 1}$$

where g and p are the numbers of ovules and pollen grains produced per unit of investment to female and male functions, respectively, and $G = g(1 - a)(1 - s)(f_1 + qf_2)$ and $P = p[a(f_1 + qf_2) + (1 - q)f_2]$ are the average numbers of ovules available for outcrossing and the average number of pollen grains produced per individual, respectively.

At equilibrium, the genotype fitnesses will be equal. Thus, setting $w_1 = w_2$ and solving for f_2 , we find that the equilibrium frequency of the plastic phenotype is

$$f_2 = \frac{r + 2s\delta - rs - 2}{2(1 - q)(r - 1)(1 - s\delta)}. \quad \text{Eqn 2}$$

It follows from Equation 2 that $f_2 > 0$ only if $r > 2(1 - s\delta)/(1 - s) = A$, independent of q . This condition is identical to that predicted by models for androdioecy that do not take explicit account of a plastic component to gender expression. Thus, a genotype that expresses a fully male phenotype with any probability can be maintained in a population with hermaphrodites only if it successfully disperses more than twice as much pollen when it is a male as that dispersed by hermaphrodites. As established by earlier models, this twofold fertility threshold for the maintenance of androdioecy increases with the population selfing rate. It is also evident from Equation 2 that the plastic genotype will completely replace the fixed hermaphroditic phenotype in the population if $r > 2q(\delta s - 1)/[1 - 2q + s(1 - 2\delta + 2\delta q)] = B$, which also requires that $q > 0.5$. Thus $A < r < B$ defines the parameter space in which a genetic polymorphism can be maintained; this space is illustrated in Fig. 1.

Note that $m = f_2(1 - q)$ is the frequency of individuals in the population with a male phenotype – in other words,

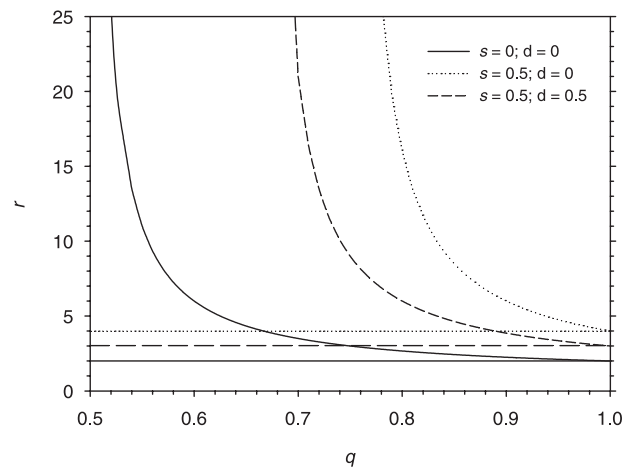


Fig. 1 The minimum value of r required for fixation of the plastic genotype 1 (i.e. the genotype with partial male expression), as a function of q , the probability that the plastic genotype has a hermaphroditic phenotype. (Note that the domain of q ranges from 0.5 to 1.0.) Populations occupying parameter space above each curve will be monomorphic for the plastic genotype (and dimorphic for gender, with male and hermaphrodite frequencies given by Equation 3). Populations occupying the space below each curve and above the corresponding horizontal lines will be segregating for the fixed and plastic gender genotypes (and dimorphic for gender, with male and hermaphrodite frequencies given by Equation 3). Populations occupying the space below the straight lines will be monomorphic for the fixed hermaphroditic genotype (and monomorphic for gender). Curves are shown for three scenarios with different selfing rates and levels of inbreeding depression (indicated).

the frequency of the plastic genotype multiplied by the probability that it expresses a male phenotype. Substituting $f_2 = m/(1 - q)$ into Equation 2 and solving for m gives

$$m = \frac{r + 2s\delta - rs - 2}{2(r - 1)(1 - s\delta)}. \quad \text{Eqn 3}$$

This is the frequency of males in an androdioecious population at equilibrium, as first derived by Lloyd (1975). It is evident that the frequency of males is independent of the extent to which gender expression is genetically fixed or plastic, so that the equilibrium condition is fully described in terms of gender by models that ignore the possibility of plastic sex expression. In particular, the same male frequency can be maintained in populations that differ widely in their value of q , because of frequency-dependent covariation in the underlying genotype frequencies (Fig. 2). Note that when $q > 0$ and $A < r < B$, there will be both a genetic and a plastic component to the variation in sex expression, with the genetic component maintained by negative frequency-dependent selection (Fig. 1). When $r > B$, the plastic genotype will be fixed and genetic variation for sex determination will thus be lost from the population (Fig. 1). However, the population will still be dimorphic in gender, and Equation 3 will still formally apply at evolutionary equilibrium.

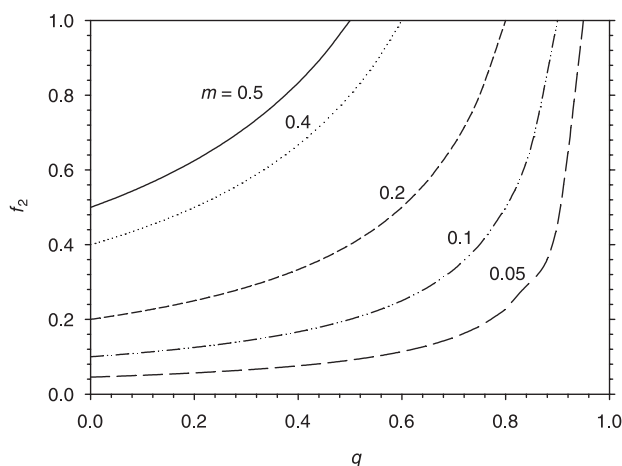


Fig. 2 The frequency of the plastic genotype, f_2 , in a population in which the plastic genotype expresses a hermaphroditic phenotype with probability q . Each curve corresponds to a population at sex-allocation equilibrium with the indicated frequency of males.

How will selection act in a population in which gender variation is entirely plastic? Assume, as before, that individuals develop as hermaphrodites with probability q and as males with probability $(1 - q)$. Let the gender of a single mutant be hermaphroditic and male with probabilities q' and $(1 - q')$, respectively. We use the standard technique to find the evolutionarily stable probability of hermaphrodite expression, q^* , by solving

$$\left. \frac{\partial w'_2}{\partial q'} \right|_{q'=q} = 0$$

and setting $f_2 = 1$. We find that $(1 - q^*)$ is equal to the solution for m in Equation 3, the frequency of males in a genetically dimorphic population comprising plastic and fixed gender genotypes. In an infinite population, the second derivative of w'_2 with respect to q' is zero, indicating that q is not locally stable: a population with average gender expression q^* can be invaded by plastic genotypes expressing any value of q in $(0,1)$. In a population of finite size, the second derivative is negative at $q' = q$, and q^* is locally stable. We may therefore conclude that the equilibrium frequency of individuals with a male phenotype in a population of hermaphrodites is independent of the extent to which genotypes may switch their gender between male and hermaphrodite phenotypes. Phenotypic descriptions of gender are thus sufficient for predicting the frequency of gender phenotypes at evolutionary equilibrium, whether or not sex determination has a genetic component.

Discussion

In their review of androdioecy, Delph & Wolf (2005) claim that plasticity of the sort explicitly recognised in the model I

have presented here 'is not part of the traditional definition of androdioecy, which is defined as having individuals that are genetically determined to be pure males' (p. 126). One may quibble about how particular terms have been defined traditionally, but the model presented here shows clearly that frequency-dependent selection will ultimately bring the phenotypic frequencies of males and hermaphrodites to rest at predictable equilibria, irrespective of their genetic or developmental basis. Delph & Wolf (2005) suggested that an analysis of sexual systems should be approached by comparing the fitness contributions of genotypes rather than of phenotypes. Equilibrium sex ratios can in fact be predicted by equating either genotype fitnesses (w_1 and w_2) or phenotype fitnesses (males and hermaphrodites). However, equilibrium models of androdioecy predict phenotype frequencies.

Of necessity, the model presented in this paper made rather specific assumptions about the determination of gender in an androdioecious population. However, it would be easy to make similar models for other situations, including the case of gynodioecy, and these models would also predict the frequency of phenotypes, irrespective of their genetic or developmental basis. It is worth noting that this applies as much to the case of gynodioecy with cytoplasmic sex determination as it does to gynodioecy with nuclear sex determination. Under nuclear sex determination, the equilibrium phenotype frequencies are found by equating the fitness gained by each phenotype through both sexual functions, as in models for androdioecy (Lloyd, 1975; Charlesworth & Charlesworth, 1978). In the case of cytoplasmic sex determination, in contrast, phenotype fitnesses must of course be evaluated with reference only to female function, which may be pollen-limited at equilibrium (Lewis, 1941). In discussing these models, we are free to refer to the respective morphs as phenotypes or genotypes, but the phenotypic perspective will be sufficient to account for gender ratios in populations at equilibrium. This general property of phenotypic models has been widely discussed elsewhere (e.g. Lloyd, 1977; Bulmer, 1994; Frank, 1998).

It is important to stress that although a genetic perspective is redundant for understanding equilibrium sex allocations, a knowledge of the genetic basis of sex determination and its potential interaction with environmental signals can be extremely valuable. For example, the genetic architecture of gender will strongly influence the shape of evolutionary trajectories in populations that have not reached equilibrium (Pannell *et al.*, 2005). The complex dynamics that account for morph frequencies in gynodioecious species with nucleocytoplasmic male sterility are a good example (Frank & Barr, 2001; Bailey *et al.*, 2003). Here, gender frequency variation is the result of nonequilibrium conditions over space and time due to mismatches between maternally inherited male sterility mutations and biparentally inherited male fertility restorer genes (Frank & Barr, 2001).

Another example concerns the relative importance of dominant vs recessive sterility mutations in the evolution of gynodioecy or androdioecy (Charlesworth & Charlesworth, 1978; Pannell, 1997). Because rare recessive advantageous mutations are more likely to be lost by drift than rare dominant ones, the early spread of a sterility mutation will depend on its dominance coefficient (Charlesworth & Charlesworth, 1978). In a metapopulation, the dominance of sterility mutations can also affect the global frequency of gender morphs at equilibrium. This is because the global morph frequency is the sum over evolutionary trajectories in populations that have not reached local equilibrium (Pannell *et al.*, 2005). Understanding these interactions involves more complex modelling and more detailed empirical knowledge than that for single populations at equilibrium (Pannell, 1997; Frank & Barr, 2001). However, the potential complexities need not influence the way we view gender a priori.

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Key words: androdioecy, gynodioecy, hermaphroditism, phenotypic plasticity, sex determination.



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