Commentary

Steering the solar panel: plastids influence development

As if they were industrial factories, plant cells reveal a multitude of interactions between compartments, with many activities requiring the traffic of components and products between them. Thirty years ago researchers made what seemed, at first, a surprising observation: a mutant of barley that, through maternal inheritance of ribosome-deficient plastids, possessed contiguous stripes of green and white tissue, showed, in the white tissue, not only very low levels of plastid-based enzymatic activities but also very low levels of synthesis template for them in the cytoplasm (Bradbeer *et al.*, 1979). The deficient state of the plastids was providing information to the nucleus of those cells, leading to low mRNA levels of the nuclear-encoded genes for those enzymes in the cytoplasm where they were synthesized. The compartments were sharing information, including commands from seemingly subordinate organelles (namely the chloroplasts), to control expression of genes in the nucleus. This specific flow of interorganellar information is known as plastid–nuclear communication or plastid retrograde signalling. In this issue of *New Phytologist*, Ruckle & Larkin (pp. 367–379) provide evidence showing that this retrograde signalling controls not just the nuclear processes involved in the biogenesis and function of the organelle, but also aspects of the differentiation of cells and the development of the plant overall in its response to light, at least at its crucial seedling stage. Light is a key environmental cue without whose presence neither chloroplast biogenesis nor normal (photomorphogenic) seedling development take place. Ruckle *et al.* (2007) recently showed that plastid signals are capable of 'rewiring' the light signalling network controlling a gene (*Lhcb1*) for a major chloroplast protein; the cryptochrome1 (cry1*) photoreceptor and a bZIP downstream transcription factor, HY5, seemed to reverse their roles in the expression of this gene if plastids were damaged. In this issue, Ruckle & Larkin show that this reversal, as judged by the consequences of the absence of cry1, applies also to several other aspects of seedling photomorphogenesis: for example, the expansion of the first photosynthetic organs

(the cotyledons) and, under some conditions, the elongation of the seedling stem (the hypocotyl), although it does not affect responses related to the production of sunscreens in nonphotosynthetic cells.

'Thirty years after this intracellular conversation was identified in plant cells, a bewildering number of facts remain shrouded in mystery.'

Plastid–nucleus co-ordination

Retrograde signalling, as observed by Bradbeer *et al.* (1979) (Fig. 1), is part of a wider set of mechanisms that ensure appropriate development and performance of chloroplasts
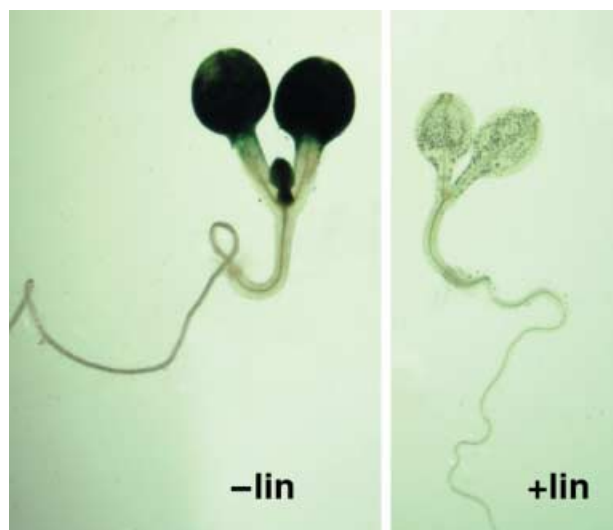

Fig. 1 Evidence for plastid retrograde signalling. *Arabidopsis* seedlings carrying a nuclear reporter construct containing an *Lhcb1* promoter driving the expression of β-glucuronidase (GUS) were grown for 10 d in white light in standard tissue culture medium (−lin) or for 2 d in standard medium and then for 8 d in medium supplemented with the plastid translation inhibitor lincomycin (+lin). The GUS activity appears as dark blue staining. The reporter is equivalent to that used as part of the *gun* mutant screening (Susek *et al.*, 1993).

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* It is convention in Arabidopsis to refer to photoreceptor holoproteins, with chromophore attached, in non-italised lower case, therefore we refer to cry1 as the holoprotein.

within photosynthetic cells (recently reviewed by Pogson *et al.*, 2008). When photobleaching of plastids occurs, or when plastid protein translation is impaired, the synthesis of nuclear-encoded polypeptides for many plastid proteins ceases. The generation of reactive oxygen species in the plastid also has impacts on nuclear gene expression, which are diverse, depending on the reactive species, for plastidic as well as for detoxifying, cytoplasmic proteins, and even initiates cell death; this is not simply a passive toxicity effect because it requires specific proteins (suitably named EXECUTERS, Lee *et al.*, 2007). Meanwhile, at least in algae, the synthesis of plastid subunits of complexes is co-ordinated with that of their partners encoded in the nucleus by means of 'epistasy of synthesis', by which plastid translation is sensitive to the presence of unassembled polypeptides. Finally, even after initial development, redox imbalances resulting from unequal light excitation of photosystems are sensed and initiate, via kinase cascades, homeostatic gene-expression changes in both the plastid (Puthiyaveetil *et al.*, 2008) and the nucleus (Bonardi *et al.*, 2005). The signalling cascades of retrograde signalling proper are incompletely understood, but important insights began with the identification of '*genomes uncoupled*' (*gun*) mutations, which partly uncouple the expression of nuclear genes, such as *Lhcb1*, from plastid dysfunction (Susek *et al.*, 1993). A major breakthrough has been the identification of GUN1, a protein associated with plastid DNA (nucleoids) and that belongs to a family, several of whose members regulate organelle translation; evidence shows that it integrates signals from a variety of stimuli and initiates the repression of nuclear photosynthetic genes (Koussevitzky *et al.*, 2007). The extent to which photo-oxidation and plastid translation defects share a signalling pathway, as well as the nature of the 'photo-oxidation' signalling molecule, have been a matter of conflicting evidence in the literature (Cottage *et al.*, 2007; Koussevitzky *et al.*, 2007; Mochizuki *et al.*, 2008; Moulin *et al.*, 2008).

Light and plastid signals intertwining

One approach used in an attempt to unravel the plastid retrograde signalling pathway was identification of the promoter regions it targets. A key outcome of this approach was that it was found to be impossible to separate the target elements that mediate plastid and light regulation (Kusnetsov *et al.*, 1996). Koussevitzky *et al.* (2007) demonstrated that ABI4, a transcriptional repressor that mediates much of the response dependent on GUN1, binds a CC**AC** element that overlaps the **AC**GT G-box element, mediating the light response for many photosynthesis-associated genes, including *Lhcb1*. In this manner, plastid dysfunction would prevent the light response. However other minimal elements have been identified that do not share this architecture, yet they can recapitulate, in gain-of-function experiments, both the light response and the plastid dependency (Acevedo-Hernández

et al., 2005). It is evident that these two signalling pathways are central regulators of chloroplast development and are very closely related. The extent to which they are so became dramatically evident with the identification, by Ruckle *et al.* (2007), of *cry1* as a *gun* mutant. In its wild-type form, cry1 is a nuclear protein and the main photoreceptor responsible for perceiving blue light. Although *cry1* was a weak *gun* mutant on its own, double *cry1 gun1* mutants showed almost complete *Lhcb1* independence of plastid status. It is interesting to note that the presence of cry1 had also been shown to be essential for a form of programmed cell death induced by singlet oxygen generation in plastids in the light (Danon *et al.*, 2006). Importantly, under normal conditions of normal chloroplast function, cry1 is an activator of photosynthetic gene expression and chloroplast development, whereas under conditions of plastid stress it was the absence of cry1 that stimulated *Lhcb1* expression. This could be interpreted as the recruitment of different partners converting a transcriptional activator (the downstream HY5) into a transcriptional repressor. Larkin & Ruckle (2008) described this broadly as a 'gas and break' system that adjusts the synthesis of at least some chloroplast proteins to the prevailing light and plastid functional status. Ruckle & Larkin now show that the expansion of the seedling's first photosynthetic organs, the cotyledons, is under similar control: it is promoted by cry1 in the light when plastids are functional, but repressed by cry1 when plastids are dysfunctional.

Plastids affect development

In their scrupulously carried out experiments, Ruckle & Larkin showed that a similar, 'switchable' control by cry1 also applies to the hypocotyl, although only under certain conditions. The plastid functional status therefore appears to have a relatively broad developmental influence, and plastid dysfunction in general represses photomorphogenesis, at least for photosynthetic organ development. Some degree of repression of photomorphogenesis had also been observed in mutants with impaired plastid development (Vinti *et al.*, 2005).

In retrospect, this developmental influence is not surprising. It has been observed repeatedly that mutations which affect chloroplast differentiation also prevent the normal differentiation specifically of leaf palisade cells (Bellaoui & Gruissem, 2004) and that the developmental response of such cells to high light is severely impaired, in a cell-autonomous manner, by plastid dysfunction (Fig. 2). Although not highlighted as such, one of the most spectacular observations of Ruckle & Larkin is the fact that the differentiation of guard cells in the epidermis of cotyledons is influenced by plastid signals: in both *gun1* and *cry1* mutants plastid dysfunction greatly reduces the expansion of epidermal cells, but only in the *gun1* mutant does this cause a large increase in the density of stomata, implying that under certain conditions, complete cell-fate decisions can be under organellar control.
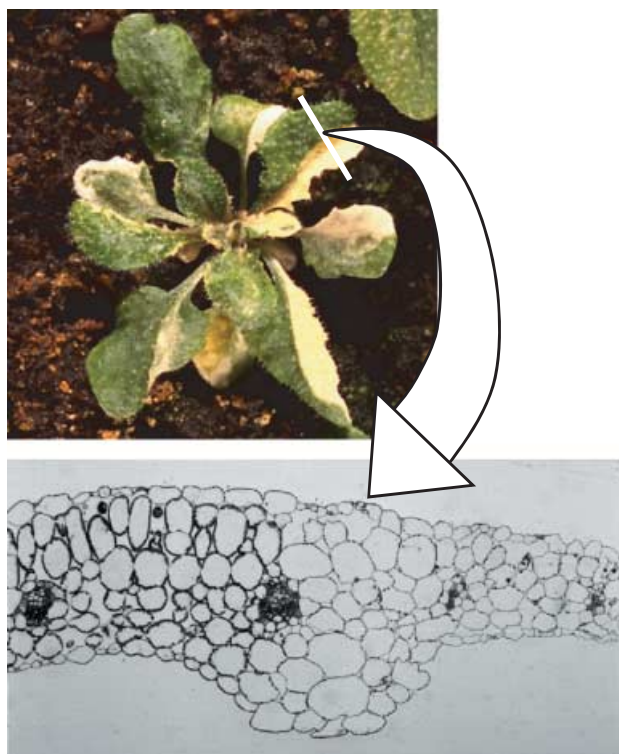
Fig. 2 Developmental, cell-autonomous effects of the plastid status. A leaf of the variegated *chm1* mutant, fully expanded under high fluence rate white light (600 μmol m^{-2} s^{-1}), was fixed and sectioned for microscopic analysis. Palisade cells on the green sector (left of the section) appear elongated, whereas those in the white sector are less so, and the number of cell layers is greater in the green sector, implying a plastid function requirement for cell elongation and cell division. Adapted from Tan *et al*. (2008).

Is plastid signalling built upon pre-existing light signalling or *vice versa*?

The concept of 'rewiring' provides a beautifully simple evolutionary design principle for the action of plastid signals. The impact of the signals dependent on GUN1 would feed into a pre-existing network that utilized HY5, a positive regulator of many light-responsive genes. Conversely, it is worth noting that a form of plastid retrograde signalling, one in which the nuclear gene for a heat shock protein depends on plastid stress signals (von Gromoff *et al*., 2006), occurs in *Chlamydomonas*, a single-celled green alga in which chloroplasts do not require light to develop. The plastid stress signals are both chlorophyll precursors and haem in this case. It is difficult to gauge whether this pathway is orthologous to any of those that control *Lhcb1* in flowering plants. It has, however, been shown that plastid retrograde signalling is functional in the absence of COP1, a ubiquitin ligase that targets HY5 for degradation and thus acts as a central repressor of photomorphogenesis in the dark (Sullivan & Gray, 1999). COP1 belongs to an ancient class of conserved

eukaryotic regulators of basic cellular processes. The control of chloroplast development by light, meanwhile, is likely to be relatively recent, because many gymnosperms, like pine, while showing photomorphogenic responses, exhibit greening in the dark and light-independent expression of *Lhcb1* (Yamamoto *et al*., 1991). It would be interesting to explore which of these two signalling processes controlling chloroplast development preceded which, in evolutionary terms, and this would also help to reconstruct the intricacies of their interaction.

Does plastid signalling act as an emergency break, or as a start-up cut-out?

The existence of *gun* mutants provides genetic evidence most-parsimoniously consistent with plastid signals being active repressors (i.e. 'distressed' plastids produce negative signals that repress photosynthetic nuclear genes) and loss of the *GUN* genes relieves this repression. This is the conceptual framework for the 'break' metaphor of Larkin & Ruckle (2008). One could argue that the *gun* mutants are, to plastid retrograde signalling, equivalent to what the *de-etiolated* or *constitutively-photomorphogenic* mutants are in relation to light signalling. Of course, the existence of the latter does not imply the absence of active photoreceptors. This is an interesting issue, but one very difficult to tackle experimentally. Do distressed plastids produce repressive signals, or do functional plastids produce positive signals that are lost when plastids malfunction? In fact, the answer could be affirmative for both questions. While chloroplasts with accumulated, photodynamic chlorophyll precursors, like those of the *flu* mutant (Lee *et al*., 2007), can be easily conceptualized as sources of repressive signals, the lack of photosynthetic gene expression when plastid development is severely reduced, for example when aminoacyl-tRNA synthetases are lacking and plastids barely differentiate (Barkan & Goldschmidt-Clermont, 2000), is less easily so. Would such plastids contain highly active, repressive GUN1? While activity would be difficult to measure, the levels of GUN1 in such plastids could easily be. It is hard, however, to imagine how to address experimentally this ultimate nature of plastid signalling except by examining the gene expression status of cells devoid of plastids, functional or otherwise – certainly a challenging task.

Thirty years after this intracellular conversation was identified in plant cells, a bewildering number of facts remain shrouded in mystery. Clearly, a cell is more than the sum of its parts, and the complexity of the exchange of information between those parts does not cease to amaze us.

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Bidirectional water flows through the soil–fungal–plant mycorrhizal continuum

In the autumn droughts when soils are powdery dry, when fires rage across the landscape, when the grasses have cured and the forbs have dried and blown away, and even deep-rooted trees and shrubs are drought stressed, *Pisolithus* sp. fruits in the most inhospitable dry soils, through cement sidewalk cracks and between rocky outcrops (Fig. 1). Fruiting of some of these mycorrhizal fungi poses one of the most intriguing questions in these drought-plagued regions. The new paper by Erik Lilleskov and his colleagues in this issue of *New Phytologist* (pp. 483–494) takes the step of demonstrating the importance of the complex transport of water from deep in the soil to the mycorrhizal sporocarps. Understanding the results presented in this paper, and the laboratory and field studies on hydraulic redistribution preceding it, underscores the dynamic and important complex structural elements that actually comprise the soil–fungal–plant interface.

'... that Amanita muscaria *may even parasitize the water of* B. edulis *is intriguing, only adding to the complexity of organism interactions occurring despite the drought conditions ...'*

Fig. 1 Sporocarps of *Pisolithus* sp. associated with *Chrysolepis sempervirens* fruiting during the late summer drought.

The summer of 2005 saw some of the most severe droughts ever recorded in Europe. Climate projections from the Intergovernmental Panel on Climate Change (IPCC) and other sources show increasing drought in southern Europe, the southwestern USA and northwestern Mexico, the Sahel, South Africa and Australia. Understanding drought stress responses by plants and their symbiotic partners, from the atmosphere into the bedrock, is the basis for the careful management of fire intensity, drought and marginal land production. Research on the plant–atmosphere interface of the soil–plant–atmosphere continuum (SPAC) has made major advancements over the past few decades to the point where predictions from satellite data are relatively accurate. Predictability breaks down, however, at the soil–plant inter-face. Much of the work remains based on irrigation technology, with a focus on saturated flows under high levels of soil water, and the work is often based on diffusion models and on laboratory-measured flow properties. However, in unsaturated soils, flow models become highly complex because of the microscale variation in soil pores and in solid materials, in dynamic roots and in even more dynamic microbes. Work on mycorrhizas, particularly over the past decade, has altered our understanding of the mechanisms whereby mycorrhizal fungi and their host plants exchange resources, and the complex array of ever-shifting water flux directions, flux rates and interdependencies (Allen, 2007). Stahl (1900) first postulated that water uptake is a major regulator of mycor-

rhizal functioning. Little did he anticipate how complex that process actually was! Mycorrhizal fungi not only cover the portion of the root where absorption of nutrients predominates, but create an extensive and dynamic mycelial network ranging far from the root tip and even into the bedrock.

Water moves in response to energy gradients. As soils fill with water as a result of rain or snowmelt, first small pores, and then large pores, fill, creating a high water content (θ). That water absorbs nutrients and salts, creating an energy difference from the comparatively pure rainwater. This difference in energy is called the osmotic potential (ψ_π). Plants extract water from the soil at a rate equal to the transpiration from the leaves, first from the larger pores. As it is depleted, water in the pores forms a thin meniscus along the surface of the soil particles, organic micelles and soil organisms and is left in ever-smaller pores. As roots extract water from these pores, and the larger ones are depleted, gradients in water form between the filled pores and the emptied pores. The energy to extract that water bound to the soil particles is called the matric potential (ψ_m). These gradients are expressed in units of energy (mega-pascals, MPa) as the energy difference between two solutions on opposite sides of a membrane or between two locations. Water potential is a function of the concentration of solutes (ψ_π), the adhesion to a surface (ψ_m) and gravity (ψ_g).

Water flow from soil to the leaves is also dependent upon adhesion between water molecules forming a hydraulic continuum from the soil pores through the plant and into the atmosphere (Fig. 2). Any break, or cavitation, can result
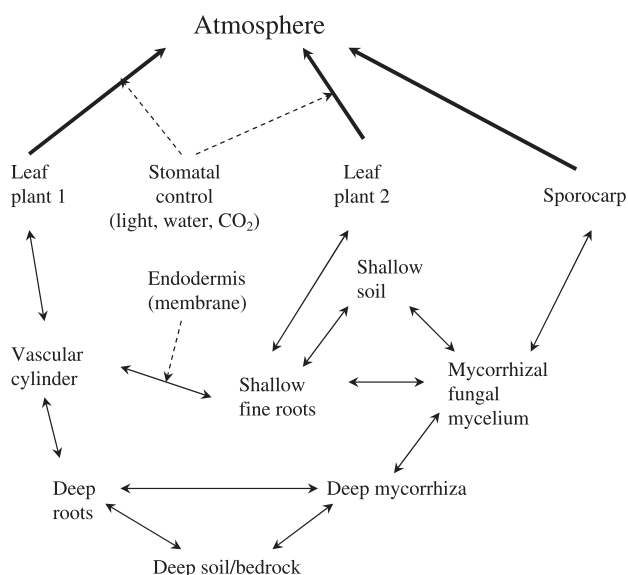

Fig. 2 Water flows and hydraulic redistribution in Mediterranean-type ecosystems during the late summer drought. Bidirectional flow is indicated by bidirectional arrows, and those flow rates and directions are dependent upon the water potential gradients between the two locations.

in the death of the plant. In an irrigated cropland or mesic environment, water in the macropores (i.e. those > 80 μm) is extracted by roots and then replenished by irrigation or precipitation. However, in arid lands, that replenishment often occurs infrequently. Water retreats back to smaller pores (mesopores). Just as importantly, as soils dry to that of the wilting point (−1.5 MPa), roots shrink, creating air gaps between the root surface and the soil particles. Root hairs, if present, can penetrate the mesopores (down to 30 μm), but rarely smaller. In Mediterranean and semi-arid climates, the proportion of the growing season where soil dryness is lower than the wilting point can exceed 40% of the year, including most of the year when soil temperatures support growth. Thus, the portion of the growing season when the root surface is not directly in contact with soil water can be high. Mycorrhizal fungal hyphae bridge these air gaps, and rhizomorphs and individual hyphae radiate into the smaller soil pores, including micropores, with gaps of only a few mm^3. This is one mechanism whereby mycorrhizas can increase water uptake from the surface soils (Allen, 2007). This remaining soil water becomes even more concentrated in solutes, including nitrogen (N) and phosphorus (P), and becomes inaccessible to roots; eventually even the hyphae should dry out. However, growth and metabolism continues in the many evergreen plants and their mycorrhizal fungi. The question is, how?

Tracking water movements has been aided by important technologies not available until recently. Dyes have become far more specific, and isotopic tracers facilitate the identification of important transport mechanisms. Just as importantly, the use of natural-abundance isotopes allows researchers to quantify transport rates in the field, not just to postulate mechanisms based on glasshouse chamber studies. However, these methods require extensive calibration of potential sources and modeling of differential fractionation to be carried out during the transport and transpiration process. Lighter isotopes (H and ^{16}O) transpire more rapidly than heavier isotopes (D and ^{18}O).

In the course of these studies, researchers have found that in many arid environments, plants (especially shrubs and trees) develop deep roots that penetrate into fractures in the bedrock and even form mycorrhizas with fungi that penetrate decomposing rock material (Egerton-Warburton et al., 2003; Bornyasz et al., 2005), extracting deep water (you can get water from a stone!). In southern California and eastern Oregon, woody evergreen plants require access to this water stored in granite or they would die from drought because there is not enough soil water to sustain them through the dry season (Sternberg et al., 1996; Warren et al., 2007).

Another process was discovered when studying the importance of deep water, namely hydraulic redistribution (Richards & Caldwell, 1987). In hydraulic redistribution, the water is transpired normally during the day; however, at night, when stomata close, if the surface soils have a ψ that is lower

than that of the vascular tissue, water flows horizontally into fine roots. If there were no mycorrhizas, then the water would diffuse into the air gaps surrounding the shrunken roots. However, most of these roots are mycorrhizal. With their intimate contact with dry soil, the mycorrhizal fungal hyphae and soil have a more negative ψ than does the plant vascular system. Because of the lower ψ, this redistributed water is transported along and into the hyphae (Querejeta et al., 2003), and even into a neighboring root, if that root has a compatible mycorrhiza (Egerton-Warburton et al., 2007; Warren et al., 2008).

While small amounts of water are needed to sustain the hyphae of mycorrhizal fungi (Querejeta et al., 2007, in press; Warren et al., 2008), Lilleskov et al. demonstrated that, in fact, rather large amounts of water can move by this mechanism – so much so that it can create enough osmotic force to push sporocarps through crusts, rock and cement fractures, during the most extreme dry season.

Lilleskov et al. found that particular fungi have adapted this mechanism for sporulation, particularly *Boletus edulis*, whereas others with less well developed rhizomorphs (*Russula* spp.) did not show this extensive use of deep water. Their finding that *Amanita muscaria* may even parasitize the water of *B. edulis* is intriguing, only adding to the complexity of organism interactions occurring despite the drought conditions of these highly diverse ecosystems.

In California, *Pisolithus* sp. and *Rhizopogon* sp. both form rhizomorphs, and both were found forming ectomycorrhizas deep in the decomposing granite bedrock (Egerton-Warburton et al., 2003). Along with *B. edulis*, these fungi are also common among semi-arid woodlands and Mediterranean-type habitats worldwide. Access to deep water directly, or by hydraulic lift, has the potential to alter dramatically how we think about phenology and mycorrhizas in semi-arid regions of the globe, as well as forestry and agriculture in marginal lands. Simple assumptions, such as nutrient acquisition being limited to periods of wet soils, the importance of dry deposition of N to the soil surface, and soil respiration and carbon fluxes during the dry season, will all take on new dimensions as the physiological activities of mycorrhizal hyphae during hydraulic lift are further explored.

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Key words: deep roots, drought, hydraulic lift, mycorrhiza, sporocarp
production, water.

Striking example of avatars evolving together among local communities

An 'avatar' is a local population of a species in a local
community; it is the local embodiment of the group – the
tangible representative that interacts with avatars of other
species (Damuth, 1985). In this issue of *New Phytologist*,
Anderson & Johnson (pp. 533–540) report on a striking
story of avatars evolving under the influence of one another
in diverging communities. Previously, Anderson & Johnson
(2008) reported on a species of long-tongued fly, *Prosoeca
ganglbaueri*, which seems to have co-evolved locally with a
species of Scrophulariaceae, *Zalusianskya microsiphon*. In the
area studied, the fly gets much of its nectar from this one
plant; the scroph is even more specialized in being pollinated
almost exclusively by this one fly. The fly's average tongue
length varies among sites from 20 to 50 mm, and the scroph's
average floral tube length covaries from 19 to 55 mm. The

positive covariation is strong and significant even after taking
into account factors such as body size and elevation. Further-
more, the variation is a geographic mosaic rather than being
clinal. The fly and the scroph together have undergone extreme
divergence among populations within their respective species.

'The cool thing about P. ganglbaueri *and its flowers is
that tongue and tube lengths are jazzing around within
species, among populations, across their geographic
ranges, not subsequent to the establishment of a phylo-
genetic scaffold.'*

The story is wondrously more complicated than just
these two species. A third species is an orchid named *Disa
nivea* that offers no nectar reward (Anderson *et al.*, 2005). It
depends exclusively on *P. ganglbaueri* for pollination. It is the
same color as the scroph, and its avatars have diverged among
sites, along with the scroph, in floral tube length and in flower
width. The orchid is a Batesian mimic, and the scroph is its
rewarding model. Experiments confirm that the mimic suffers
reduced pollination success when it is placed in a high-density
patch without many inflorescences of the model compared
with when the rewarding scroph is present at high density
(Anderson & Johnson, 2006). In the current paper, a reciprocal
transfer experiment was carried out in which orchids with
long and short tubes were moved between communities that
had long vs short tongues and tubes.
• For pollinaria removed (a measure of male function), there
was an interaction in which the long-tubed orchids had more
pollinaria removed at a site where tongue lengths and floral
tubes were long, whereas short-tubed orchids had more polli-
naria removed at a site where tongues and tubes were short.
• For receipt of pollen (a measure of female function), there
was no interaction, just large parallel main effects in which the long-
tubed orchids were better pollinated at both sites, and flowers
were better pollinated at the site where everything was long.

All this is consistent with a scenario in which the avatars of
the orchid have been evolutionarily tracking the divergence
among local populations in the tongue length of the flies,
perhaps with the selection being sexual selection on the
efficiency of pollen transfer.

However, there are not just these three species involved.
Anderson & Johnson also found *Gladiolus oppositiflorus* at a
number of the sites studied, and its flower depth correlated
tightly with fly tongue length. In fact, there are *c.* 20 species

of flowers that are pollinated by *P. ganglbaueri*. Most of them are rare and probably have relatively little effect on the fly's evolution compared with the effect of the common scroph. However, the fly seems to have driven the divergence of floral tube lengths among populations of the rare species. For each of the rare species found at more than one site, the functional tube length varied significantly, and often in the direction of being longer when the fly's tongue was longer. Not all of these flowers are specialists on only *P. ganglbaueri*, and one would expect that the way in which populations diverge ought to be a complicated response to the interactions with all their pollinators. Nevertheless, placing all the species together on one scatterplot reveals a general correspondence between fly tongue length and the depth of floral tubes among populations.

Pay attention for a moment to the scroph–fly mutualism. What exactly are the selection mechanics that drive their organs to become so long, and the length to become so varied, among populations? The explanation dating back to Darwin (1862) hinges on assuming that flowers that are just a little bit longer than the going-rate for fly tongues have enhanced pollen transfer – the longer tube would force pollinators to make closer or more precise contact with the sex organs of the flower as they sip nectar from the depths of the corolla tube. This would constitute selection for ever-longer scroph tubes. Because the fly relies specifically on the scrophs of the local population for much of its food, there would also be selection for tongues to be longer rather than shorter. The two partners would always be engaged in a runaway process (Wallace, 1867; Nilsson, 1998; cf. Wasserthal, 1997). That process might be held in check by just how costly it is to the fly to have such a long tongue protruding from its face and/or the cost of growing such a long nectar tube, or the degree to which populations are pollinator limited. The costs might vary from site to site, depending on environmental conditions, such as how windy the site is. Also, populations might have been stalled in the runaway process at varying stages by the lack of new mutations that lengthen the organisms' organs.

The runaway process would not be so coupled if both scroph and fly were less specialized; also, if they did not depend specifically on one another, their geographic patchiness would not coincide, which might fail to foster so much local divergence. The degree of specialization is unusual for systems of flowers and flower foragers. More often either the flower, or the forager, would be a generalist (Waser & Ollerton, 2006). Thus, we should not use *P. ganglbaueri* and its flowers to exemplify pollination mutualisms. Rather, the system is placed towards one extreme. As yet we have no details on how the system came to be so specialized, although southern Africa seems to have more than its share of specialized pollination systems that have evolved in-place with relatively little community reassembly (Johnson & Steiner, 2000). Even though the story of *P. ganglbaueri* and its flowers must be understood in the context of the principal actors being specialists, the phenomenon of local adaptation to pollinators causing a geographic

mosaic in flowers does not depend on extreme specialization (Thompson, 2005). Generalist flowers experiencing a varying mosaic of pollinator communities presumably adapt to the local pollinator mixes they experience (Dilley *et al.*, 2000), but the story of generalists wandering evolutionarily seems harder to document as the history seems harder to infer.

Because of the specialization involved, one is tempted to draw a parallel between the phenomenon of local pollination ecotypes radiating evolutionarily and another phenomenon much discussed in pollination biology, namely that of shifts between pollination syndromes. Think of bee-pollinated flowers giving rise to hummingbird-pollinated flowers. Indeed, Anderson & Johnson introduce their paper by citing work on pollination syndromes. As they imply, the two phenomena are related, but I would point out that there could also be a marked difference. *P. ganglbaueri* and its flowers exemplify differences in organ length arising among many populations within species, organ length being a character that is evidently free-to-vary in flies and in flowers of several species. But shifts between pollinators probably occur late in the process of species divergence, as a matter of multitrait co-adaptation in just one of the lineages emerging from cladogenesis, and shifts seem to be relatively rare events on flower phylogenies (Whittall & Hodges, 2007; Thomson & Wilson, 2008). It may well be true that all-adaptation-is-local, but it is certainly not the case that all differences arise at the cutting edge of divergence. Two incipient species may come to be adapted to different elevations first, and then because of an unusual ecological community, one of the lineages may shift to a new pollinator and away from its ancestral adaptive norm. The cool thing about *P. ganglbaueri* and its flowers is that tongue and tube lengths are jazzing around within species, among populations, across their geographic ranges, not subsequent to the establishment of a phylogenetic scaffold. In each very local community, the several avatars have been evolving, quite possibly co-evolving.

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Letter

Are true multihost fungi the exception or the rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co-occurring *Quercus* species

Most ectomycorrhizal (EM) plants associate with many unrelated ectomycorrhizal fungi (EMF), and many EMF associate with several different plant hosts (e.g. 'multihost' or 'generalist' fungi; Bruns *et al.*, 2002; Kennedy *et al.*, 2003). This generalist approach may enhance plant success, because seedlings dispersing into diverse habitats may find compatible EMF and may therefore have a competitive advantage (Bruns *et al.*, 2002; Nara & Hogetsu, 2004). Association with many EMF may also enhance a plant's access to nutrients, particularly if certain EMF are adapted to unique microhabitats or have access to different nutrients (Baxter & Dighton, 2001). Roy *et al.* (2008) estimated that multihost EMF comprise between 12% and 90% of all species, and suggested that multihost EMF increase the chance of carbon transfer between different hosts, even when present in low frequencies. Although multihost EMF dominate many ecosystems (Horton & Bruns, 2001; Selosse *et al.*, 2006), there are also important specialist taxa restricted to distinct host plant groups. EMF host specificity has been well documented in several genera, including *Leccinum* (den Bakker *et al.*, 2004), *Strobilomyces* (Sato *et al.*, 2007), *Alnicola* (Moreau *et al.*, 2006), *Alpova* (Nouhra *et al.*, 2005), *Chondrogaster* (Montecchi & Sarasini,

2001), *Terfezia* and *Tirmania* (Díez *et al.*, 2002), and many Suillineae (*Suillus*, *Rhizopogon*, *Truncocolumella* and *Gomphidius*) (Bruns *et al.*, 2002). It is also well established that some individual EMF species have strong host preferences (e.g. *Lactarius obscuratus* – Molina, 1979; *Tuber melanosporum* – Murat *et al.*, 2004; *Tricholoma matsutake* – Lian *et al.*, 2006), although these taxa may or may not be truly host specific.

To better understand host effects on EMF communities, we have previously documented the EMF as sporocarps and on root tips of *Quercus douglasii* Hook & Arn. and *Quercus wislizeni* A. DC. (Smith *et al.*, 2007; Morris *et al.*, 2008). These *Quercus* species are dominant, endemic EM trees throughout a large area of interior, low-elevation woodlands in California (Pavlik *et al.*, 1991). *Quercus douglasii* is deciduous and usually has a sparse litter layer, whereas *Q. wislizeni* is evergreen and generally has deep litter. We hypothesized that these differences in physiology and litter deposition between the two oaks would influence their EMF communities. The same dominant EMF species occurred on both *Quercus* hosts, yet, paradoxically, the overall communities were distinct. *Quercus wislizeni* showed a greater diversity and frequency of EMF with epigeous sporocarps, whereas Ascomycota were more frequent on *Q. douglasii* (Morris *et al.*, 2008). Thus, despite the close phylogenetic relationship between these plants, each hosted a unique EMF community. Multihost EMF were detected on both hosts, but some EMF taxa or functional groups apparently 'preferred' one host over the other.

Across most of their range, these *Quercus* species co-occur with another widespread endemic, *Pinus sabiniana* Douglas (Graves, 1932). These three species are the dominant EM hosts across a geographically large area, but no studies have yet examined the EMF of *P. sabiniana*. The objective of this study was to determine the dominant EMF on *P. sabiniana* roots

and to compare them with the extensively documented EMF on the co-occurring *Quercus* species. We wanted to examine whether multihost EMF would be frequently shared among the three hosts, or whether pine-preferring EMF would be dominant on *P. sabiniana*. Several recent studies have shown that plant host identity strongly influences EMF communities and that this effect is exacerbated with increasing phylogenetic distance of the hosts (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008). As the closely related *Q. douglasii* and *Q. wislizeni* had unique EMF communities, we hypothesized that the EMF community on the distantly related *P. sabiniana* would be even more divergent.

EMF sampling of *P. sabiniana* was conducted at the site studied by Smith *et al.* (2007) and Morris *et al.* (2008). The UC Sierra Foothill Research and Extension Center is located in a low-elevation woodland (400–600 m) with a strong Mediterranean climate in Yuba County, CA, USA (39°17′N, 121°17′W). We sampled EM roots on 1 April 2005 from eight randomly selected *P. sabiniana* < 50 m from previously sampled *Quercus* plots (Smith *et al.*, 2007; Morris *et al.*, 2008; 'plot 2'). *Pinus* were small to large trees found *c.* 2–15 m from *Quercus* within the contiguous mosaic of woodland–savanna. We intended to sample *P. sabiniana* as in Smith *et al.* (2007) because we assumed that *Pinus* roots would be easily distinguished on the basis of size and color. However, these differences were not obvious and preliminary sampling yielded many roots that could not be unambiguously assigned to host genus. Therefore, one lateral root system per tree (main root > 3 cm in diameter) was randomly selected and cut at the base. We then excavated the root system and extracted several 50–200-cm root sections. Each extracted root system was placed in a plastic bag, stored at 4°C and processed within 5 d following the protocols of Smith *et al.* (2007) with minor amendments. We were unable to gather 100 EM roots for two trees, and so we used all root tips for these samples (pines 1 and 6 had 82 and 32 EM tips, respectively). Tuberculate EM roots formed by *Rhizopogon* were large relative to other EM tips, and so several EM tips per tubercule were added to the pooled root tips from that sample. Taxon naming and molecular analyses of roots followed Smith *et al.* (2007), except that 94 instead of 48 clones were restriction fragment length polymorphism (RFLP) screened from each sample.

Morris *et al.* (2008) collected samples based on focal trees, whereas Smith *et al.* (2007) collected samples from a plot with many trees. In this study, we used the individual samples; although some autocorrelation may be present for samples taken around focal trees, this should not affect the host preference aspects of the data. EMF species' composition data were analyzed using principal component analysis (PCA) and multiple-response permutation procedures (MRPP) to collapse the large EMF dataset into groups and illustrate the separation of EMF communities by host. PCA depicts differences in species' composition in relation to host, whereas MRPP is a nonparametric procedure that uses randomization to test the

significance of pre-assigned variables (e.g. tree species). PC-ORD v. 4.20 was used to calculate the PCA axes based on correlation coefficients with the Sørensen distance measure, because it performs well with presence–absence data (McCune & Mefford, 1999).

We have previously sampled EMF at this site by extensive surveys of sporocarps and EM roots (*Q. douglasii*, 110 root cores with *c.* 11 000 roots; *Q. wislizeni*, 32 root cores with *c.* 3200 roots) (Smith *et al.*, 2007; Morris *et al.*, 2008). In this study, we sampled eight *P. sabiniana* (717 EM roots and 752 clones; mean of 89.6 roots per tree). We detected 33 EMF species, with two to nine species per lateral root (mean of 6.1 species). Basidiomycota were dominant on *P. sabiniana*, with 25 species accounting for 77.6% of the relative frequency (Table S1, see Supporting Information). We detected eight Ascomycota, but these only accounted for 22.4% of the relative frequency, and only two Ascomycota were detected on more than one *Pinus*. By contrast, 37–39% of the EMF species on *Quercus* were Ascomycota, and they accounted for 31–49% of the relative frequency. Furthermore, many dominant *Quercus* EMF were Ascomycota (e.g. *Cenococcum*, *Tuber*, *Peziza*). Nineteen of the 33 EMF detected on *P. sabiniana* (*c.* 58% of the taxa, 65% of the relative frequency) had not been detected previously on *Quercus*, despite sampling > 140 root cores (14 000+ roots). This was true for seven of the 10 most common *Pinus* EMF, including all species that occurred on three or more *Pinus*. Five species in two lineages that associated only with *Pinus* (*Rhizopogon*, Atheliaceae) were major contributors to this pattern; together, these taxa accounted for 28% of the relative frequency on *Pinus*. PCA visually segregates the EMF communities by host plant, although there is significant overlap between *Quercus* species (Fig. 1). MRPP confirmed
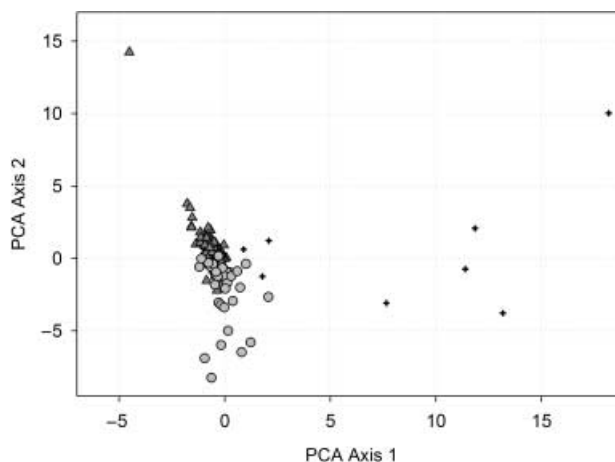

Fig. 1 Principal component analysis (PCA) of ectomycorrhizal (EM) fungal species composition relative to host tree species, showing axis 1 and axis 2. Each point on the graph represents an independent sample of EM roots from one of the three plant host species (triangles, *Quercus douglasii*; circles, *Quercus wislizeni*; crosses, *Pinus sabiniana*). (Root cores for *Quercus* spp., lateral roots for *Pinus sabiniana*.)

the separation (*t*-test; $P < 0.0001$), indicating greater variance between than within groups. To ensure that these host preferences were not strongly affected by the small *P. sabiniana* sample size, we performed 10 additional MRPP tests with reduced *Quercus* datasets. For each MRPP test, we randomly selected data from 10 *Q. wislizeni* and 10 *Q. douglasii* cores, and analyzed them with the entire *P. sabiniana* dataset. In each of the reduced datasets, MRPP confirmed the separation between the *Quercus* and *Pinus* ECM communities ($P < 0.001$), but the effect size remained low ($R < 0.10$). These low effect values are not surprising considering the smaller sample size and the nature of community data, yet the statistical analysis still confirmed higher homogeneity within groups than between. This pattern of host structured ECM communities was detected regardless of whether or not species of the Pinaceae specialist genus *Rhizopogon* were included in the PCA.

Although the majority of *Pinus*-associated EMF were only detected on *Pinus*, 14 EMF co-occurred on the roots of at least one *Quercus* species (*c*. 42% of taxa, 35% of relative frequency); all EMF species shared by both *Pinus* and *Quercus* were Basidiomycota. Five 'generalist' EMF were found on all three hosts (*c*. 15% of taxa, 10% of relative frequency). *Pinus sabiniana* shared about the same number of EMF with *Q. douglasii* (nine) as with *Q. wislizeni* (10). However, most of the EMF detected on both *Pinus* and *Quercus* tended to have low relative frequency on *Quercus* (< 2% relative frequency). Two notable exceptions, *Laccaria bicolor* (4.3% relative frequency on *Q. wislizeni*) and Thelephoraceae (3.5% relative frequency on *Q. douglasii*), may be strong 'multihost' fungi. Species of *Laccaria* may be preadapted as 'multihost' EMF because they are often pioneer species, regularly establish new genets from spores, are readily cultured in the laboratory and have great potential to become invasive (Gherbi *et al.*, 1999; Kropp & Meuller, 1999; Fiore-Donno & Martin, 2001; Díez, 2005; Roy *et al.*, 2008; Vellinga *et al.*, 2009).

Many of the higher level EMF lineages detected on *P. sabiniana* were the same as those on *Quercus* (e.g. Thelephoraceae, Pezizales), but, at the species level, many EMF were different on the two host genera. Although based on a small *Pinus* sample, these data suggest the possibility that some EMF groups may be more or less likely to exhibit host preferences. For example, Pezizales appeared to be strongly structured by host; they were common on *Pinus* and *Quercus*, but none of the seven *Pinus*-associated pezizalean EMF species were ever detected on *Quercus*. Interestingly, we detected a similar pattern of host structuring for pezizalean hyphae at the same site (M. M. Hynes *et al.*, unpublished). By contrast, other groups appeared to be less likely to exhibit host preference. For example, four of five Russulaceae were found on both *Pinus* and *Quercus*. This result for Russulaceae is similar to that presented by Ishida *et al.* (2007) where, of the 22 species that occurred more than once, 17 Russulaceae were found on more than one host plant species. These patterns of host preference may be system specific, however, as many studies have indicated that some Russulaceae exhibit strong host preferences and some Pezizales are host generalists (e.g. Hutchison, 1999; Kennedy *et al.*, 2003; Ishida *et al.*, 2007; Twieg *et al.*, 2007; Tedersoo *et al.*, 2008). Although it would be interesting if some EMF lineages tended to have stronger host affinity than others, this hypothesis requires further testing.

To date, we have detected 225 species of EMF from sporocarps and EM roots of three host species in this dry woodland habitat. This is closely comparable with the 205 EMF species reported by Ishida *et al.* (2007) from a mesic, closed-canopy forest containing 12 host species in six genera. In terms of community composition, however, the *Pinus* EMF community in this dry woodland was similar to other dry, inland *Pinus* EMF communities (Bidartondo *et al.*, 2001; Hubert & Gehring, 2008) and to the post-fire spore bank EMF community in coastal *Pinus muricata* (Taylor & Bruns, 1999). It was dominated by species with inconspicuous sporocarps, and the hypogeous genera *Rhizopogon* and *Tuber* were abundant and species rich. We only detected 10 species with epigeous sporocarps (29% of the relative frequency), and only two of these (*Hygrophorus* cf. *gliocyclus*, *Clavulina* cf. *cristata*) inhabited multiple trees. Dominant genera from mature coastal pines, such as *Suillus* and *Amanita* spp., were notably absent on *P. sabiniana*.

Unfortunately, because of the destructive techniques needed to complete this study, we were unable to sample large numbers of *Pinus* roots or individuals. With further sampling, we would probably have detected more EMF species that are occasionally shared between *Quercus* and *Pinus*. However, we believe that the overall pattern would remain; except for a relatively small number of 'multihost' taxa, most of the dominant *Pinus* EMF rarely associate with or have a low frequency on *Quercus*, and most dominant *Quercus* EMF rarely associate with or have a low frequency on *Pinus*.

The prevailing ideas about EMF host preference may be changing. Early studies of Northern Hemisphere ecosystems, mostly dominated by Pinaceae and with routinely overlapping canopies, reported high diversity and abundance of shared EMF taxa between hosts, suggesting that 'multihost' fungi are dominant (Horton & Bruns, 2001; Horton *et al.*, 1999, 2005; Cullings *et al.*, 2000; Kennedy *et al.*, 2003; Richard *et al.*, 2005; Twieg *et al.*, 2007; Hubert & Gehring, 2008) However, on a global scale, most EM host plants and ecosystems remain unstudied or undersampled (Dickie & Moyersoen, 2008). Sampling EMF communities with different sets of host plants (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008) and in different habitats (e.g. tropical forest – Tedersoo *et al.*, 2007; woodland–savanna – Morris *et al.*, 2008) might change our view of host preference and, consequently, the perceived importance of 'multihost' fungi. The emerging picture is one in which many EMF appear to display some level of host preference, and most EM plants host some fraction of the locally available EMF. These complex interactions appear to be governed by some combination of host plant relatedness (Ishida *et al.*,

2007; Tedersoo *et al.*, 2007), plant life-history traits and physiology (Morris *et al.*, 2008; Tedersoo *et al.*, 2008) and successional strategies of both hosts and fungi (Horton *et al.*, 2005; Twieg *et al.*, 2007; Roy *et al.*, 2008). The end result is that 'multihost' EMF fungi may be much less dominant than previously thought.

Acknowledgements

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Key words: ectomycorrhizal fungi, host preference, *Pinus*, *Quercus*, specificity.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Ectomycorrhizal fungi detected on the roots of *Pinus sabiniana*

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Meetings

Planning for connections in the long-term in Patagonia

Establishing a long-term ecological research program and research collaborations in northwestern Patagonia. A workshop in San Carlos de Bariloche, Argentina, January 2009

The relict flora of Gondwana, the mystic nature of the windswept Patagonian steppe, the Andes mountains and the southern beech forests, all combined, made San Carlos de Bariloche the perfect setting for gathering researchers from North and South America to discuss a common goal: establishing a program of research and scientific interaction focused on long-term ecological questions in northern Patagonia. The 4-d workshop was funded by the National Science Foundation of the USA, and by the local scientific agencies of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and was led by Barbara Bond (Oregon State University, USA), Claudio Ghersa (Universidad de Buenos Aires, Argentina) and Tomás Schlichter (Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina). The meeting served as a rare opportunity to bring together a group of high-caliber scientists, ranging from population and community ecologists to ecosystem ecologists, plant physiologists and hydrologists, all of whom had a keen interest in exploring the possibilities of developing an integrated research program in the northern Patagonian region.

In the USA, the Long-Term Ecological Research (LTER) program was initiated in 1979–80 and currently has 26 active sites in various ecosystems in North America. As a result of the consistent funding support received from the National Science Foundation during this time frame, it has been possible to conduct studies at these sites with the intention of exploring long-term research topics that would be informative to the general scientific community. Mark Harmon (Oregon State University, USA) has been working within the LTER program from its inception and provided valuable insight into the lessons learned and pitfalls to be avoided in trying to develop a new long-term research program in the Patagonian region. For example, having an LTER program is not just about long-term measurements – for real success in a program, he suggested that research priorities must be directed around
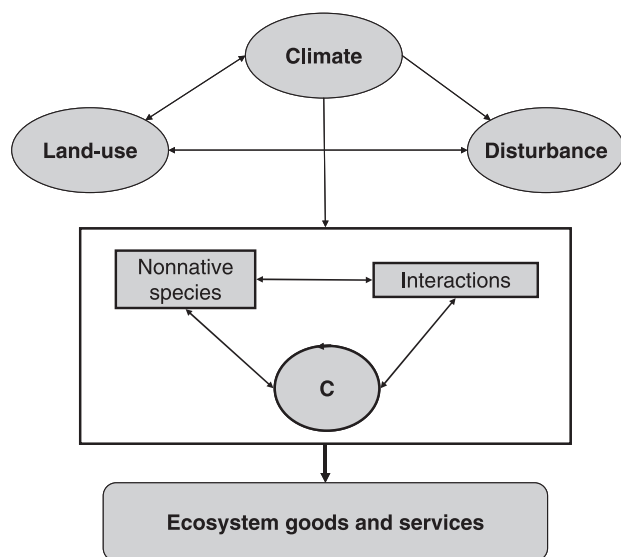
Fig. 1. Conceptual framework of priorities for long-term research in northern Patagonia. The combination of steep continuous environmental gradients of temperature and precipitation within a matrix of land-use change allows for the exploration of important impacts of the drivers of climate, land-use and disturbance in a range of ecosystems within the region. The introduction of nonnative species, novel interactions among species and trophic-level interactions, and the consequences for the carbon cycle, were identified as key topics that should be a focus of research for understanding the consequences of these drivers for ecosystem goods and services.

umbrella questions that link various ideas in a common long-term context. But the question put to the participants in the workshop was essentially this – is there the possibility of implementing an LTER program in Patagonia? The answer was a resoundingly hopeful yes, with extensive discussion to identify the initial steps required to create a conceptual framework, which would unite multiple disciplinary interests to address critical ecological questions for the region (Fig. 1).

'How functionally vulnerable and resilient are Patagonian ecosystems in the face of global environmental change and what are the consequences for ecosystem services?'

Gradients in all their glory

Northern Patagonia is unique: there was universal agreement in this workshop that one of the most outstanding features of the region is the steep orthogonal climatic gradients of temperature and precipitation. The combination of the influence of topography and consistent climatic patterns generates a gradient of decreasing rainfall from the Andean mountain range to the Argentinean Atlantic coast. In the region from 40° to 55°S, there is a strong east–west precipitation gradient, with a shift in vegetation, in less than 150 km, from xeric desert shrubland to grass-shrub steppe, ecotones of forest-steppe leading to a low-stature tree cover and finally closed canopy forest (Austin & Sala, 2002). This rainfall gradient, combined with the latitudinal and altitudinal temperature gradients, provides the opportunity to examine a very wide range of ecosystems occurring in close proximity in a matrix of climatic conditions.

A second aspect of the region that is extraordinary is the evolutionary and biogeographic heritage of the flora and fauna in northern Patagonia. The links to austral (Gondwanan) floral species, including members of the Proteaceae and of the dominant *Nothofagus* species in Patagonian forests, demonstrates the evolutionary isolation of the region. Combined with this heritage, evidence including congeneric relationships with existing tropical species and a frequent occurrence of plant–animal mutualisms suggest that much of the flora of temperate South America can be considered as a reflection of a warmer past with a neotropical history (Aizen & Ezcurra, 2008). As such, the interactions among species, and the response to global change, may be very different from those found in other regions of the world owing to the rare combination of biogeographic and historical events.

Fire, tree mortality and synchronized masting events

One of the clearest results to emerge from this workshop was that there is great potential for joining the scientific efforts of a number of co-existing research programs in the region, many of which already have an explicit 'long-term' focus. Infrequent events that occur over longer time frames can have important impacts on Patagonian ecosystems. For example, studies of long-term drought effects on *Nothofagus* spp., carried out by Tomás Kitzberger's research group (Universidad Nacional de Comahue, Argentina), demonstrate that the vulnerability of tree mortality during drought events is a function, in part, of previous drought events (Suarez *et al.*, 2004). Studies of masting events in the long-lived monocarpic bamboo, *Chusquea culeou*, which flowers every 60 yr, have shown that these masting events have important impacts on predator–prey interactions and on overstorey forest regeneration (Kitzberger *et al.*, 2007; Raffaele *et al.*, 2007; Giordano *et al.*, 2009). In addition, periodic masting in dominant overstorey species, such as *Auracaria auracana* and *N. obliqua*, highlight the importance of multiple-year studies for understanding tree demography in the region (Sanguinetti & Kitzberger, 2008). Finally, Tom Veblen (University of Colorado, Boulder, USA)

has been working in Patagonia for almost 30 yr and has carried out a range of studies demonstrating the importance of fire and of climate on stand dynamics in native forest species (Veblen *et al.*, 1999). The importance of fire and its interaction with human behavior emerged as a central focus for long-term research in the region, in particular as one of the modulators of ecotone boundaries.

Where did those pines come from?

In this region of Patagonia between 39° and 42°S, one of the dominant land-uses is afforestation of exotic species, which started in the 1970s in areas ranging from arid steppe to native forest. Currently, there are over 70 000 ha under cultivation with various conifer species, principally *Pinus ponderosa* (> 90%) and, to a lesser degree, *Psuedotsuga menziesii* and *Pinus contorta* var. *latifoliada* (Laclau, 2003; Licata *et al.*, 2008). The current use of these plantations varies substantially and ranges from commercial exploitation of timber resources to maintenance of hunting and recreational habitats. Recent studies by the participants in the workshop have demonstrated substantial alterations of the hydrologic cycle as a result of pine afforestation in semi-arid ecosystems of the region (Fernández *et al.*, 2008; Licata *et al.*, 2008), carbon cycling (Nosetto *et al.*, 2006) and negative impacts on native animal biodiversity (Paritsis & Aizen, 2008). The current and future impacts of these plantations on biogeochemical cycles, including carbon sequestration, nutrient availability and biodiversity, were identified as a priority within the context of the long-term research plan.

Novel interactions

The introduction, whether intentional (as in the case of pine afforestation) or not, of a number of nonnative species, has had important impacts on the ecosystems in the region. While the effects on particular ecosystem processes or characteristics are important, a research priority emerged for this region that focused not only on the impact of the nonnative species, but on how these introductions affect interactions among species and trophic levels. For example, the research group of Marcelo Aizen (Universidad Nacional de Comahue, Argentina) recently demonstrated that the invasion of alien mutualists in the mesic forests of the region weakened the strength of the mutualisms among plants and their pollinators (Aizen *et al.*, 2008). Another recent study demonstrated a different type of interaction, that of the relationship between the litter of overstory trees and their decomposers, where an affinity exists such that decomposition of 'home-site' litter is faster than in other microsites in the forest (Vivanco & Austin, 2008). These unique interactions among species, and their potential disruption, were identified as a key vulnerability of these Patagonian ecosystems to human-induced global change.

The potential for novel human interactions was also a primary goal of this workshop. Elizabeth Borer (Oregon State University, USA) suggested that another part of the long-term research effort could be a link to their global-scale effort to establish common nutrient-manipulation experiments in grasslands worldwide (URL: http://www.science.oregonstate.edu/~seabloom/nutnet/). The Nutrient Network (NutNet) is a low-cost collaborative research effort to address the questions of nutrient limitations and their effects on biodiversity within a co-ordinated research network currently comprising more than 40 grassland sites worldwide.

The will and the way

The central research question that arose from the animated discussions was directed towards understanding and predicting the vulnerability and resilience of northern Patagonian ecosystems to global environmental change (Fig. 1). Within this scientific context, however, one must include the human dimension – not only its impact on ecosystem goods and services, but also the importance of human-induced changes in the environment. Given this positive launch of the idea of a long-term collaborative research program in the region, what is needed now is a network of interested researchers and funding opportunities, to identify future directions for establishing research networks and collaborations within the region.

The critical challenge is not the will, or the scientific potential, but the way in which an LTER program could be implemented in the region. It is difficult to obtain long-term funding for any research program, and this is more so in Argentina, where funding cycles can be erratic and consistent support over time is not guaranteed. What is clearly needed is a marshalling of resources, using multiple strategies from both national and international sources. The potential benefits of this endeavor are many and can be measured at multiple levels: (1) the gathering and dialogue of researchers with common scientific interests of conservation of natural resources in the region; (2) the potential for interactive research networks that increase the value and impact of individuals working in the area; (3) the possibility of a focal point for international scientists to participate in research projects with insights and analytical tools that are not currently available; and (4) the establishment of long-term connections among local Patagonian researchers, students, and national and international institutions. Finally, the hope is that this initiative could serve as an inspiration for other similar programs within Argentina and South America.

Acknowledgments

E. Raffaele, T. Schlicter, C. Sierra, T. Veblen, and to INTA for hosting the workshop.

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