Commentary

Progress in the detection of costs of phenotypic plasticity in plants

Among the most interesting biological phenomena is the fact that a genotype can develop different phenotypes depending on the environment in which this development takes place. Historically, however, the focus was on homeostasis by canalization of the phenotype to a presumed optimum (Waddington, 1960), and phenotypic plasticity was interpreted as deviation from such an optimum and therefore considered a nuisance. This has changed drastically with the insight that many plastic responses, such as stem elongation in response to shading, are actually adaptive strategies that increase fitness. Surprisingly, however, not all organisms are highly plastic, which suggests that the evolution of phenotypic plasticity is constrained either by a lack of heritable genetic variation or by limits and costs of plasticity, which outweigh its potential benefits (DeWitt *et al.*, 1998; van Kleunen & Fischer, 2005). In this context modelling studies (van Tienderen, 1991) emphasized the role of costs of plasticity. It is therefore very astonishing that empirical studies have found little evidence for the existence of such costs (van Kleunen & Fischer, 2005). Possibly, costs of plasticity are difficult to detect because genotypes burdened by high costs of plasticity have been purged from natural populations by natural selection (DeWitt *et al.*, 1998), and may only re-emerge after recombination (Fig. 1). This motivated Dechaine *et al.* (this issue, pp. 874–882) and two further recent studies (Callahan *et al.*, 2005; Weinig *et al.*, 2006) to test costs of plasticity with recombinant inbred lines rather than with natural plant genotypes.

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Costs of plasticity and their detection

A cost of plasticity is manifested as a reduction in fitness of a genotype as a consequence of the ability to express a certain phenotype through plastic rather than fixed development (van Tienderen, 1991; DeWitt *et al.*, 1998). In other words, if a genotype with fixed development always growing 10-cm-long leaves happens to grow in an environment where a plastic genotype also grows 10-cm-long leaves, costs of plasticity will become apparent as reduced fitness of the plastic genotype relative to the fixed genotype in this environment. This could be the result of maintenance costs of the sensory and regulatory machinery required for plasticity, less stable development of plastic genotypes and intrinsic genetic costs as a result of pleiotropy, linkage and epistasis involving genes relevant for variation in fitness and plasticity (van Kleunen & Fischer, 2005). However, to date there are no studies disentangling the relative importance of these mechanisms.

As illustrated by the example of leaf-length plasticity in the previous paragraph, a cost of plasticity is indicated by a

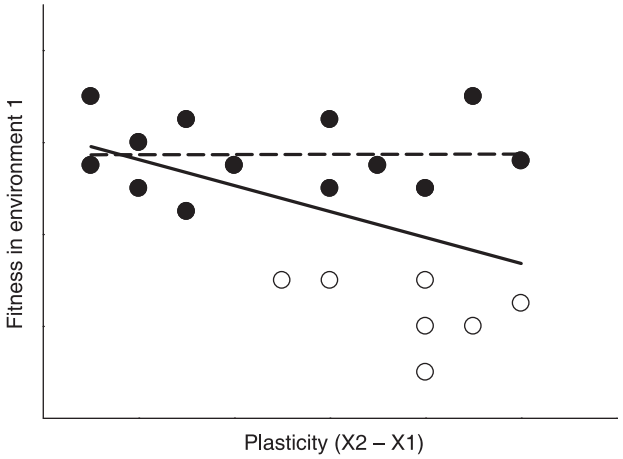

Fig. 1 Illustration of purged costs of plasticity and their re-emergence in segregating offspring. Costs of plasticity are indicated by a negative slope of the regression line of fitness in one of the test environments against plasticity in trait X across two environments. When genotypes with low fitness (open symbols) have been purged from the population, no cost of plasticity (dashed line) is found among the remaining genotypes (solid symbols). However, when the purged genotypes re-emerge in segregating offspring, a cost of plasticity (solid line) is found. Plasticity values are calculated by subtracting the mean trait value (X) of environment 1, in which low trait values are adaptive, from the mean trait value of environment 2, in which high trait values are adaptive.

negative selection gradient for plasticity (Fig. 1) when, for a large number of genotypes, fitness in one environment is related to phenotypic plasticity in an ecologically relevant trait measured by comparing the trait in two or more environments (for more details, see Dechaine *et al.*). The low percentage of cases where costs of plasticity were detected might indicate that costs are rare or difficult to detect (van Kleunen & Fischer, 2005). Alternatively, as mentioned earlier, genotypes burdened by high costs of plasticity might often have been purged from natural populations by natural selection (DeWitt *et al.*, 1998). However, even if currently purged, such costs might still constrain evolution of plasticity if they regularly re-emerge after recombination. This is especially likely for the genetic costs of plasticity that result from linkage or epistasis. Therefore, Callahan *et al.* (2005) proposed to use recombinant inbred lines (RILs) instead of natural genotypes for tests of costs of plasticity. RILs are created by crossing two parental genotypes to create an F1 offspring that is then selfed to create an F2-offspring generation. Each of the F2 offspring is then selfed for multiple generations to create (nearly) identical homozygous offspring for each RIL. The latter procedure offers the advantage that phenotypic plasticity can then be assessed at the genotype level.

Twelve previous studies using natural genotypes of plants detected costs of plasticity in 43 of 333 analyses (eight studies reviewed in van Kleunen & Fischer, 2005; Caruso *et al.*, 2006; Griffith & Sultan, 2006; Weijschede *et al.*, 2006; Avramov *et al.*, 2007). Compared with these studies, three recent studies using RILs found more evidence for costs of plasticity. Callahan *et al.* (2005) grew RILs of *Arabidopsis thaliana* that had undergone different vernalization treatments, and found significant costs of plasticity in one of four analyses (for one of two traits). Weinig *et al.* (2006) grew RILs of *A. thaliana* at low and high densities, and found significant costs of plasticity in four out of 12 analyses (for three out of six traits) and Dechaine *et al.* grew RILs of *Brassica rapa* at low and high densities, and found significant costs of plasticity in four out of 12 analyses (for three out of six traits). This suggests that intrinsic genetic costs of plasticity were purged from natural populations and re-emerged in segregating progeny. However, in comparison to most other studies on costs of plasticity, the studies by Weinig *et al.* (2006) and Dechaine *et al.* also used more stressful environments, which might have increased the chances of detecting costs of plasticity (van Kleunen & Fischer, 2005). Therefore, although the use of RILs appears very promising, it is still too early to conclude that it really increases the likelihood of detecting costs of plasticity.

Dechaine *et al.* argued that purging of intrinsic genetic costs of plasticity is most likely in highly selfing species that only rarely produce segregating offspring in which costs would reappear. Similarly, one would expect such purging also to be likely in species that predominantly reproduce clonally. However, no such pattern emerges in the 12 studies on costs of plasticity of natural genotypes of plants. Of the 114 analyses on the highly selfing *A. thaliana*, 16.7% revealed costs of plasticity. Of the 44 analyses on the highly clonal *Ranunculus reptans* and *Trifolium repens*, 9.1% revealed costs of plasticity. Of the 175 analyses on species with other reproductive strategies, including *Impatiens capensis*, *Iris pumila*, *Lobelia cardinalis*, *Lobelia siphilitica*, *Plantago coronopus*, *Picea omorika*, *Polygonum persicaria*, *Polgygonum hydropiper Raphanus raphanistrum* and *Sinapis arvensis*, 11.4% revealed costs of plasticity. Clearly, however, there are too few studies to draw any strong conclusions on differences in costs of plasticity between species of different life histories.

Benefits of plasticity or benefits and costs of homeostasis?

The studies by Dechaine *et al.* and Weinig *et al.* (2006) also serve to illustrate nicely another important issue in studies of costs of plasticity. While both mainly found negative selection gradients for plasticity, indicating costs of plasticity, they also found positive ones. Interestingly, summed over all studies of costs of plasticity in plants, significantly positive selection gradients for plasticity (45 out of 361 analyses) are almost as common as significantly negative ones (52 out of 361 analyses). As it is hard to imagine why potential plastic responses should benefit fitness as long as they are not expressed, it may be asked whether positive selection gradients reported in some studies are artifacts having come about by chance. Because positive and negative selection gradients are found with similar frequency, this could imply that negative selection gradients would also be artifacts. However, because costs of plasticity are very plausible, negative selection gradients cannot all be the result of chance effects alone. This situation draws our attention to the exact biological interpretation of positive selection gradients.

Scheiner & Berrigan (1998) suggested that a plastic increase of a trait should be as costly as a plastic decrease. Consequently, many studies of costs of plasticity used absolute values of plasticity. In such analyses, a positive selection gradient for plasticity can unambiguously be interpreted as a cost of homeostasis (Dorn *et al.*, 2000). However, it should also be considered whether the plastic response is active, in the sense that it has evolved because of its higher fitness compared with any fixed phenotype, such as increased leaf length in the shade, or whether it is passive, such as a reduction in leaf length resulting from resource deficiency (Fischer *et al.*, 2000; van Kleunen & Fischer, 2005). Because it is unlikely that the costs of a plastic trait increase and a plastic decrease are the same when the increase is achieved by an active adaptive plastic response while a decrease comes about by a passive plastic response, tests for the costs of plasticity should use signed rather than absolute values (van Kleunen *et al.*, 2000; van Kleunen & Fischer, 2005; Weinig *et al.*, 2006; Dechaine *et al.*). In such

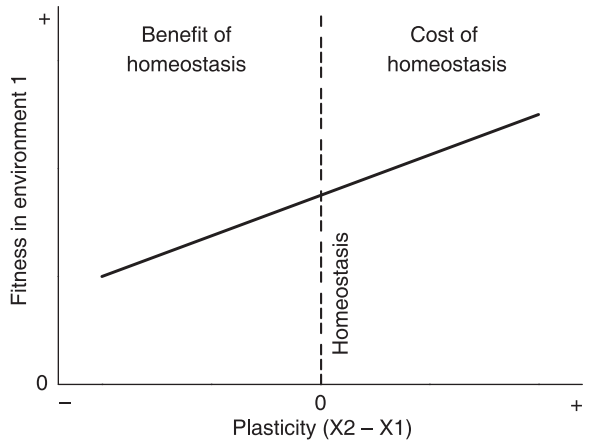

Fig. 2 Illustration of the interpretation of a positive selection gradient of plasticity. A positive selection gradient indicates a benefit of plasticity. Considered in more detail within the framework of homeostasis and active and passive plasticity (see text for further details), the interpretation of a positive selection gradient depends on whether signed plasticity values are positive or negative. A positive selection gradient indicates a benefit of homeostasis when all data points are in the left half of the graph, and it indicates a cost of homeostasis when all data points are in the right half of the graph. Note that analogous considerations apply for the interpretation of negative selection gradients. Plasticity values are calculated by subtracting the mean trait value (X) of environment 1, in which low trait values are adaptive, from the mean trait value of environment 2, in which high trait values are adaptive.

analyses, however, the interpretation of a positive selection gradient of plasticity is less straightforward. When signed plasticity values are all positive, a positive selection gradient indicates costs of homeostasis (right half in Fig. 2), as is the case for absolute plasticity values. This is exemplified by the positive selection gradient of plasticity in number of branches in response to density reported by Dechaine *et al.* However, when plasticity values are all negative, a positive selection gradient indicates that there are benefits of homeostasis (left half of Fig. 2). This appears to be the case for the positive selection gradient of plasticity in apical inflorescence height of *A. thaliana* (Weinig *et al.*, 2006). These considerations indicate that selection gradients for plasticity are of high biological relevance, but that they need to be interpreted very carefully. Clearly, to understand whether costs and benefits of plasticity really do exist, the conceptual framework of homeostasis and plasticity and the issue of active vs passive plasticity need to be considered when interpreting future selection gradient analyses. Similarly, previous studies need to be carefully reinterpreted if they did not comment on these issues.

Perspectives

Callahan *et al.* (2005), Weinig *et al.* (2006) and Dechaine *et al.* used RILs derived from only two parental genotypes, and as a consequence of this limited genetic variation, the observed costs constitute a minimum estimate of the costs that could occur in natural populations (Dechaine *et al.*). Future studies could therefore further increase the chance of detecting costs of plasticity by using segregating offspring from multiple parent pairs. Moreover, also including the parent genotypes in the experiment will allow for testing explicitly whether costs of plasticity have indeed increased in the segregating offspring relative to the parent generation.

Unfortunately, separate analyses for different traits and studies on single pairs of environments, as discussed here, may reveal only part of the picture. Therefore, among the many interesting topics that still need to be addressed in the context of costs of plasticity, potential trade-offs between plastic responses to different environmental stimuli and between plastic responses of different traits sharing the same sensory and response pathways deserve particular attention. Ultimately, this may involve insight into the exact molecular basis of costs of plasticity (van Kleunen & Fischer, 2005). Clearly, many questions remain on the evolution of phenotypic plasticity and its constraints. Studies using new approaches, such as the one by Dechaine *et al.*, are especially important, as they stimulate further progress in this exciting research field.

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Key words: active plasticity, benefits, costs, passive plasticity, phenotypic plasticity, recombinant inbred lines, selection gradient analysis.

Letters

The sunshine-mediated trigger of synchronous flowering in the tropics: the rubber tree as a study model

Synchrony of flowering is important for outbreeding trees to maximize the chances of successful pollination. This is particularly crucial for species such as the rubber tree, *Hevea brasiliensis*, which has a naturally low rate of fruit-set (Warmke, 1951; Rao, 1961) even with artificial pollination (Ghandimathi & Yeang, 1984). *Hevea* flowering promodia are formed 2–3 months before blooming (Dornelas & Rodriguez, 2005). An environmental stimulus subsequently triggers the rapid and synchronous development of preformed floral meristems to the stage of flower maturity. In Malaysia, which lies close to the equator (*c.* 3°N), the main flowering season is from February to April, while a secondary season takes place during August and September (Yeang & Ong, 1988).

The celestial trigger for synchronous flowering at the equator

If a physiological episode in a perennial plant occurred consistently at the same time every year, it is likely to be linked to some recurrent meteorological event. In the case of the rubber tree, this consistency is maintained across different rubber-growing regions with varying climatic patterns, from the humid tropics to the monsoonal subtropics that experience marked wet and dry seasons (Yeang, 2007). The signal for synchronous flowering must therefore be one that largely transcends such environmental disparities: some form of celestial cycle, for instance. One meteorological factor that escapes the influence of localized seasonal climatic conditions (other than cloud cover) is sunshine. There is a reasonable likelihood, therefore, that that synchronous flowering in the rubber tree (and other tropical trees sharing similar flowering characteristics) is connected with some aspect of solar radiation arising from the movement of the earth around the sun.

The best researched aspect of light-mediated stimulus for flowering is the photoperiod. Long-day (short-night) plants and short-day (long-night) plants flower when their photoperiod requirements are met. While these are established norms in temperate regions, photoperiod control of flowering faces a problem at the equator where day length does not vary from 12 h year-round. More than that, a stimulus linked to the change in day length (either long or short day) would imply a single annual flowering. However, flowering in tropical trees near the equator is frequently bimodal (Holtum, 1931; Borchert *et al.*, 2005), the rubber tree being one such example.

The trigger that is sought for equatorial synchronous flowering must therefore be not only a sunshine-mediated factor that is independent of day-length variation, but also bimodal in its cycle. The search for such an environmental stimulus has yielded two candidates: the bimodal advance in sunrise–sunset times measured against the chronometer, and the bimodal variation in solar radiation intensity.

Bimodal cycles of sunshine at the equator

The tilt of the earth's axis relative to the sun gives rise to a seasonal photoperiod variation that regulates flowering in

many temperate plant species. Unlike in temperate regions where incoming solar radiation (insolation) is dependent on both the day length and the radiation intensity, insolation at the equator is due entirely to the latter. At the equator, insolation peaks twice a year at the equinoxes, when the midday sun is directly overhead. In my previous paper in *New Phytologist* (Yeang, 2007), I noted that rubber trees growing near the equator and in the subtropics flowered when solar radiation intensity was high. I inferred from this that the cycle of solar radiation intensity was responsible for synchronous anthesis and blooming in *Hevea* and some other tropical trees.

When the earth's elliptical orbit around the sun is superimposed on to the tilt of the earth, the cyclical change in sunshine becomes even more complex. A discrepancy from chronometer time arises in the time-keeping that is determined from the position of the sun. One such effect is the bimodal variation in sunrise–sunset times that forms the basis of another hypothesis to explain synchronous flowering at the equator. Borchert *et al.* (2005) proposed that the seasonal shifts in sunrise–sunset times as a result of the earth's axial tilt and its elliptic orbit provided meteorological signals for flowering at the equator. By the sunrise–sunset advance hypothesis, tropical plants are triggered to flower around the time the sunrise or sunset advances are fastest in spring and autumn.

The discussion that follows looks at how each hypothetical sunshine-mediated signal might function to induce synchronous flowering in the tropics.

Character of the light signal in the sunrise–sunset time-shift model

Whatever the nature of the light signal that regulates flowering, the plant has first to detect some facet of the sunshine that it receives. The classic phytochrome photoreceptor has been used to explain light signalling while cryptochromes and phototropins are other classes of photoreceptors that have emerged more recently (Briggs & Olney, 2001; Mockler *et al.*, 2003). Light-activated genes in plants typically respond to some qualitative aspect of the light signal, such as its spectral composition (e.g. red: far-red light, blue light), or to a quantitative aspect, such as the duration or intensity (Searle & Coupland, 2004; Ausín *et al.*, 2005). Unlike seasonal photoperiod change in temperate regions, the sunrise–sunset time-shift model does not invoke change to the 12 h photoperiod at the equator. Neither does it involve change to any other qualitative or quantitative aspect of the light signal, such as its duration, intensity, direction and spectral quality. The only change is to its timing. Hence, the difference in light signal that the plant perceives would be neither qualitative nor quantitative in its nature. It would be essentially temporal. The photoreceptor does not sense what has changed or how much has changed, but when the change (sunrise or sunset) takes place.

The light signal might act directly to induce gene transcription on its own, or indirectly as a trigger to set off a cascade of reactions in the flowering pathway. Direct action seems unlikely, given that time shift of an otherwise unaltered light signal would not provide the same opportunity as a qualitative or quantitative change to induce substantial gene transcription. If the light signal were a trigger that facilitated or favoured certain cell reactions in a manner comparable to the photoperiod control of flowering (Putterill *et al.*, 2004; Ausín *et al.*, 2005; Bäurle & Dean, 2006; Zhou *et al.*, 2007), the plant would need to integrate the sunrise or sunset advance into its endogenous circadian cycle. Indeed, the sunrise–sunset hypothesis proposes that the plant measures sunrise and sunset times against its circadian clock to trigger flowering (Borchert *et al.*, 2005). In this connection, therefore, it is pertinent to examine how the circadian clock might operate at the equator.

To set and regulate its innate circadian cycle, the plant takes its cues from the solar day. Essentially, the circadian clock entrains itself to solar time. Since day length does not change at the equator, the intervals between sunrise, noon and sunset are constant year-round. Thus, even as the timing of noon drifts forward or backwards seasonally relative to chronometer time, sunrise and sunset move in tandem. It does not matter at which instant in the solar day (whether it is sunrise, noon, sunset or any point in between) that the plant uses as the reference for the entrainment of its circadian cycle, because there is only one solar clock running at the equator. While solar time is conventionally measured by the passage of the sun across the meridian at noon, it is equally well defined at the equator by the timing of sunrise on the eastern horizon or sunset on the western horizon.

Yet if the shifts in sunrise–sunset, on the one hand, and the plant's circadian clock, on the other, are both referenced against solar time, they cannot be out of phase and cannot be discrepant with each other. How, then, might a time shift in sunrise or sunset superimpose on the plant's circadian clock to register a signal for flowering? At the equator, the sunrise-sunset cycle *is* the circadian cycle.

In formulating the sunrise-sunset advance hypothesis for synchronous flowering, the notion of chronometer time is brought into the picture. The plant is thought to detect small cyclical discrepancies that arise between solar time and chronometer time. Since sunrise and sunset at the equator lie in the same time-frame as noon, which defines solar time, that obliges the plant's circadian cycle to follow chronometer time for the discrepancy to exist and for the hypothesis to stand. This rather untenable proposition prompted my earlier comment (Yeang, 2007) that 'gradual time shifts are meaningful only when measured against an external reference chronometer'. Chronometer time is a concept of anthropogenic engineering. Plants do not have an awareness of the precise chronometer time integral to the hypothesis (until the 18th century, neither did people).

Character of the light signal in the solar radiation intensity model

As the solar radiation intensity hypothesis of flowering provides for quantitative changes to the light signal that the plant perceives, functionality of the signal is not necessarily dependent on an interaction with its circadian cycle. Strong sunshine may play a more direct role in the transcription of genes that either promote floral development or relieve its inhibition.

If high solar radiation induced synchronous flowering, might there be a threshold intensity that is reached and exceeded before the trigger is actuated? Alternatively, might an increasing trend in solar radiation intensity be the critical criterion, analogous to the increasing or decreasing photoperiods reported for various tropical and subtropical plant species (Rivera & Borchert, 2001; Rivera *et al.*, 2002; Borchert *et al.*, 2005)?

In temperate regions, there is an almost 6-month increasing trend in the light photoperiod culminating in the summer solstice, followed by a 6-month decreasing trend towards the winter solstice. Therefore, when long-day plants flower before the summer solstice, it is always when day length is ascendant. Similarly, short-day plants typically flower when day length is on a declining trend. But at the equator, each period of solar radiation increase or decrease is only half as long since the annual cycle is bimodal. Thus, there are two 3-month periods of increase in sunshine intensity, culminating in the equinoxes, and two 3-month periods of decrease, culminating in the two solstices. The argument against the requirement of an increasing solar radiation trend is that the flowering season of species such as *H. brasiliensis* straddles the insolation peak. In Malaysia, *Hevea* flowering commences in February when solar radiation is on the increase. However, new floral buds continue to emerge and develop to anthesis even in April, after the equinox, when insolation is, in fact, decreasing. These observations are therefore more consistent with the explanation of a threshold insolation having been reached or exceeded.

The overhead sun makes the case for flowering being induced by high solar radiation intensity at the equator. Yet the variation in seasonal radiation need not necessarily be entirely quantitative in nature; it could be qualitative as well. When the midday sun is directly overhead, it passes through a relatively thin layer of the earth's atmosphere and the sunlight that reaches the ground is close to full-spectrum white light. Sunlight that is beamed in at an angle (e.g. at sunrise and sunset) and has thus to pass through a thicker layer of atmosphere is subjected to a greater degree of Rayleigh light scattering by gaseous molecules in the air. Such light scattering loss is more severe for the shorter wavelengths, blue, indigo, violet and ultraviolet. Besides the diurnal variation, there is also a seasonal cycle of spectral difference in light scattering at the equator since the midday sun is directly overhead only at the equinoxes. Therefore, the rubber tree flowers at the time it receives the full dose of blue-UV light from the overhead sun, and when the blue : red light ratio is maximal. There should also be a discrepancy between red and far-red light, although this would be relatively smaller because the difference in wavelengths is less. (Rayleigh scattering intensity is inversely proportional to the fourth power of the wavelength.) Future work might therefore take into account both the quantitative aspect of solar radiation (its total intensity) and the qualitative aspect (its spectral composition) in view of important roles that red light and blue light play in the flowering process (Bagnall & Hangarter, 1996; Guo *et al.*, 1999; Mockler *et al.*, 2003).

Timing of synchronous flowering with increasing latitude

Hevea brasiliensis demonstrates extraordinary robustness and adaptability that allows its cultivation to span more than 20° in latitude from the equator. Although there have been recent germplasm introductions, almost all the world's established plantings of rubber can be traced to a small number of seeds from the original collection by Wickham in 1876 (Tan, 1987). Not only is rubber that is cultivated in diverse regions generally derived from the same gene pool, but the clones grown at the equator are frequently the same ones planted in the subtropics. Therefore, flowering of the rubber tree at the extremities of its cultivated range offers an uncommon opportunity to learn how synchrony in this regard is achieved.

As already noted, synchronous flowering near the equator occurs around the equinoxes when the midday sun is directly overhead. Observations on the rubber tree indicate that flowering is delayed with increasing latitude from the equator (Yeang, 2007). This is consistent with the solar radiation intensity hypothesis, as it allows for the time lapse the sun takes to migrate from the equator to the Tropic of Cancer to the north and the Tropic of Capricorn to the south. In making a similar observation of flowering delay with latitude, van Schaik *et al.* (1993), noted that flowering in various plant species growing in locations between 20–25° north and south of the equator 'closely tracked the march of the sun'.

The difference in latitudes between a rubber planting area close to the equator (e.g. Kuala Lumpur, Malaysia, 3°N) and one close to the Tropic of Cancer (e.g. Hainan, China, 20°N, or Tripura, India, 24°N) would predict a delay of *c.* 2 months using the latitudinal position of the midday sun as the reference. Why, then, is the observed delay only 1–1.5 months (Yeang, 2007), local environmental influences notwithstanding? It should be remembered that, at the equator, seasonal variation in solar radiation is dependent entirely on the angle of the sun, with the day length playing no role. For this reason, the seasonal curves for noon insolation (Fig. 1a) and total day insolation (Fig. 1b) are identical at

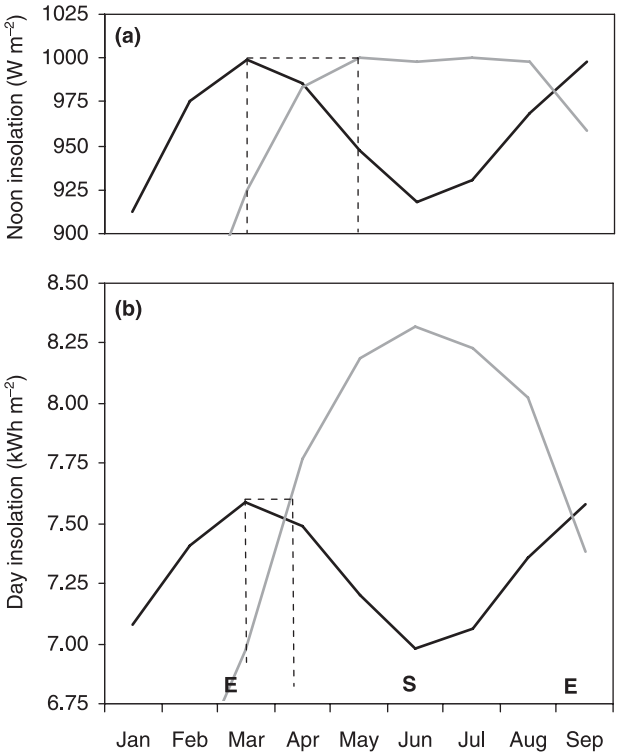

Fig. 1 Seasonal variation in solar radiation at the equator and at 20°N. Noon insolation (a) and total day insolation (b) at the equator (black line) and at 20°N (grey line) are calculated for the middle of the months as described previously (Yeang, 2007). The broken lines show insolation peaks at the equator, and the times when similar amounts of insolation are experienced at 20°N. The equinoxes (E) and the summer solstice (S) are indicated.

the equator. With increasing latitudes, however, day length begins to exert its influence and contributes to the total solar radiation received. While maximum noon insolation at the equator and at the Tropic of Cancer is essentially identical at the respective times of the year when the sun is directly overhead (Fig. 1a), total day insolation in the latter rises much higher with the advent of summer (Fig. 1b). Hence, comparable amounts of total day insolation at the equator and the tropics of Cancer and Capricorn are experienced ahead of comparable amounts of noon insolation (comparing Fig. 1a and b). This explains why flowering in the higher latitudes occurs ahead of predictions based strictly on the latitudinal position of the sun. Nevertheless, it does not necessarily mean that the entire extended photoperiod contributes towards the flowering trigger at the higher latitudes. As prolonged but weak sunshine may be ineffective in inducing flowering (Yeang, 2007), the added impact may only come from the portion of the extended day length when the sunshine is sufficiently strong. Hence, the discrepancies between the timing of flowering at the equator and at higher latitudes probably lie between what is shown in Fig. 1(a) and (b).

With the sunrise–sunset advance hypothesis, latitude increase is also expected to affect the predicted timing of synchronous flowering. Flowering at the equator is observed around the time that the rate of sunrise–sunset advance is at its peak. The maximal rate of advance, *c.* 20 s d^{-1}, is attained towards the end of March for the spring flowering. With increasing latitude, however, this same rate of advance occurs earlier (see Fig. 1 in Brochert *et al.*, 2005). Reading from the sunrise tables of the US Naval Observatory (2006) for latitude 20°N, the sunrise advance of 20 s d^{-1} would have been attained between late January and early February, well ahead of the March–April main *Hevea* flowering season in Hainan or Tripura. Thus, whereas the sunrise–sunset time-shift hypothesis predicts an advance in synchronous spring flowering with increasing latitude, a delay is in fact observed within the species, as for *Hevea*, or among various species (van Schaik *et al.*, 1993).

The sunrise–sunset time-shift hypothesis can be considered not just from the aspect of sunrise time shifts, but also from sunset time shifts (Brochert *et al.*, 2005). As already mentioned, any time advance or delay in sunrise at the equator would be accompanied by a corresponding advance or delay in sunset. Hence, there is essentially no difference whether it is the sunrise or sunset that is being monitored for the time-shift hypothesis at the equator, as they shift in tandem by equal intervals. However, this is no longer the case at the higher latitudes when day length is taken into account. It might be pertinent to recapitulate that even at locations close to the equator where day-length variation just begins to be perceptible, the photoperiodic cycle is unimodal. The longest duration of daylight falls on the summer solstice, just as it does in temperate regions.

At 20°N, the effect of increasing day length is quite significant by March and April, when rubber trees growing at this latitude flower. Even as sunrise continues to advance (i.e. the sun rises progressively earlier), the increasing light duration between sunrise and sunset means that sunset is increasingly delayed. Unlike at the equator, sunrise and sunset no longer shift in the same direction. Thus, whereas the sunrise–sunset time-shift hypothesis predicts a rapid advance in the time of sunset when the rubber tree flowers, this occurs only near the equator, but not at higher latitudes. In the latter, a delay in sunset is observed instead during the main *Hevea* flowering season in spring.

Comparing the sunrise–sunset time-shift model with the solar radiation intensity model, it can be seen that the former explains synchronous flowering near the equator, but not at the higher latitudes. The problem here lies with day-length variation at the higher latitudes. The increasingly early sunrise and the increasingly late sunset that is experienced as summer approaches confounds the prediction of flowering time. In comparison, the solar radiation hypothesis accommodates day-length variation and is operational both at the equator and in the subtropics. Indeed, prediction of flowering

time is improved in the subtropics when the day length is factored into the calculation.

Perceiving degrees of bright sunshine

While delving into how plants might sense seasonal changes in sunshine, it is also of interest to consider how the same changes are observed, or not observed, from the perspective of the researcher.

Sunrise and sunset times vary by up to 30 min over the course of the year at the equator. However, since synchronous flowering at the tropics occurs not when the sunrise–sunset advance is greatest, but when the advance is fastest, the difference between the timing of sunrise–sunset at flowering and the maximum extent of the sunrise–sunset time shift is only *c.* 15 min. This difference (1% over 24 h) is understandably difficult for the observer to notice. In comparison, the levels of noon solar radiation intensity between the minima at the solstices and the maxima at the equinoxes are of the order of 10% at the equator. Shouldn't that discrepancy have been easier to spot?

There are various explanations as to why synchronous flowering at the equator has not previously been linked to seasonal solar radiation, chief among them the contemporary temperate bias in plant physiology research (Renner, 2007). In temperate regions, seasonal differences in temperature and day length are marked, and they determine the planting cycle in agriculture. In these regions, the equinox is when the durations of day and night are equal. In the tropics, on the other hand, the planting cycle tends to be synchronized with the rains, as neither temperature nor sunshine is limiting. Equality of day and night attracts no attention where there is hardly any day-length variation to begin with. The true significance of the equinox for equatorial regions is that it is the time when there is a peak in sunshine intensity. However, that sunshine intensity varies at all over the year may not even be obvious to the casual observer at the equator.

With the five human senses at our disposal, we do not hear, taste or smell sunshine. We feel the warmth of sunshine, but not its brightness. That leaves us with the sense of sight. However, we have difficulty differentiating between degrees of bright sunshine because our eyes are equipped with a light-compensating mechanism to optimize sight in dim or bright light. When light is limited, the iris of the eye dilates the pupil fully to maximize the entry of light. As it becomes brighter, the iris constricts the pupil progressively, and in the process makes it difficult to distinguish between 'bright' sunshine and 'very bright' sunshine.

Without the aid of instrumentation set up for the purpose, the human eye may not readily discern that, at the equator, the equinox is the brightest time of the year. Plants lack eyes (although irises are found in the plant kingdom!), but they have evolved various photoreceptors capable of perceiving a broad range of light qualities and intensities. Compared with humans, plants probably do a better job of perceiving the fine degrees of bright sunshine. It could be this ability that facilitates the induction of flowering when the threshold brightness is attained.

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Key words: pollination, rubber tree, solar radiation intensity model, sunrise-sunset times-shift model, synchronous flowering, tropics.

Meetings

Functional genomics and ecology – a tale of two scales

Linking physiological ecology, evolutionary biology and functional genomics for understanding biotic responses to a changing environment and Mechanistic underpinnings of ecological processes: scaling from genes to ecosystems

Symposium and Organized session at the Ecological Society of America (ESA) 92nd Annual Meeting, San Jose, California, USA, August 2007

Science moves forward in small steps, punctuated by an occasional leap. Many believe that the advent of high-throughput sequencing of plant and animal genomes, coupled with the development of microarrays for transcript profiling, may prove to be such a leap for the biological sciences. Molecular biologists are currently using these technologies to reveal the dynamic nature of cells and organisms (Colebatch *et al.*, 2002). These advances hold equal promise for the ecologist who is willing to extend the use of these tools into the natural environment (Jackson *et al.*, 2002). Such efforts could lead to an improved understanding of how genes shape the structure and function of terrestrial ecosystems and how those insights could help us better predict the response of plants and animals to biotic and abiotic stresses in a rapidly changing world.

Two symposia were recently held at the 2007 meeting of the Ecological Society of America to evaluate the current use of functional genomics in the ecological sciences. One symposium focused on linking physiological ecology, evolutionary biology and functional genomics for understanding biotic responses to a changing environment. A second symposium addressed the mechanistic underpinnings of ecological processes with a special emphasis on scaling relationships from genes to ecosystems. The co-organizers of these symposia sought to tackle three cross-disciplinary objectives.

(1) How do we identify genes that underlie ecologically important adaptive traits?

(2) What climatic and edaphic forces will drive evolution in future, novel, environments?

(3) How do we scale from genotype to phenotype and beyond, to ecosystems?

'It is true that the ecologist will frequently have to work at the suborganismal level. The stated goal, however, should remain both integrative and extrapolative.' (Boyd Strain, Duke University, NC, USA)

Identifying genes responsible for natural variation in adaptive traits

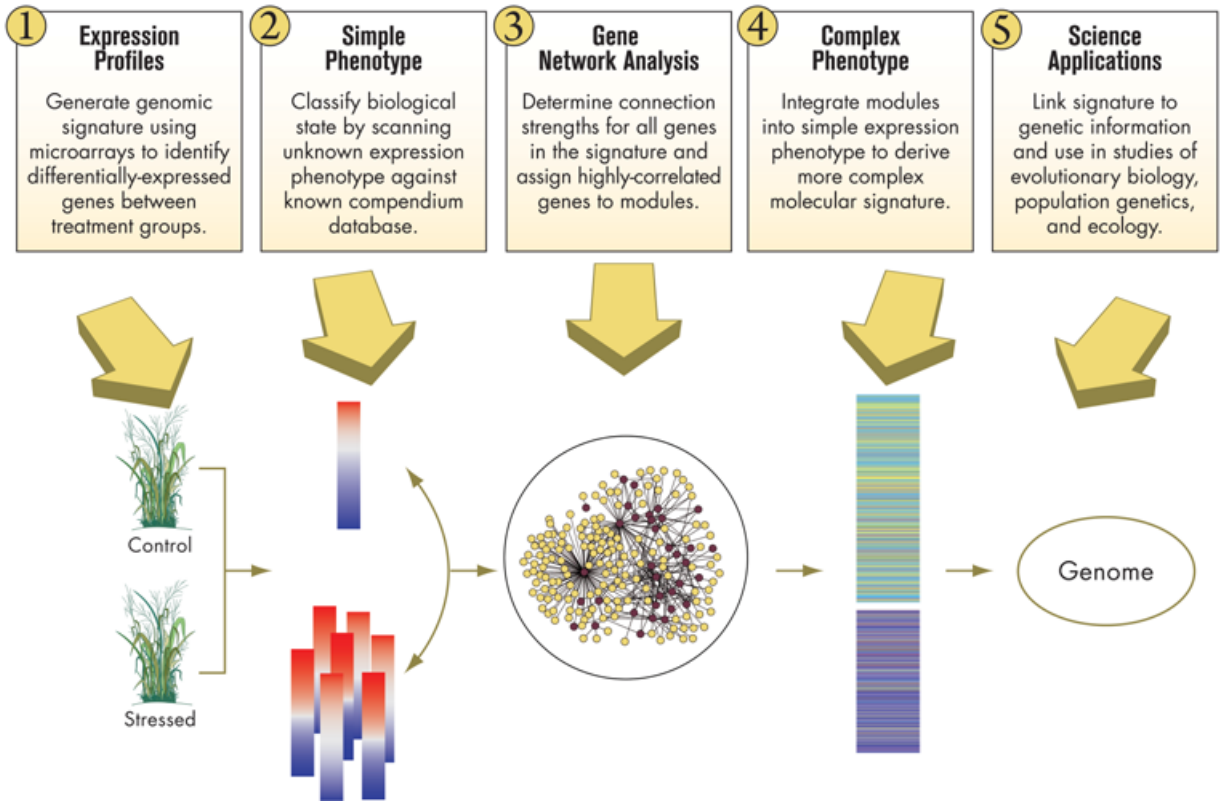
A central challenge in evolutionary and ecological genomics has been to identify the genetic basis of adaptive traits that allow an organism to survive and reproduce in natural environments (Feder & Mitchell-Olds, 2003). This challenge has been made less daunting by the increasing number of genome sequences and genetic resources that have become available in recent years. Investigators are constructing genetic linkage maps for species of interest, establishing

Box 1. Microarrays, Genomic Signatures, and More...

A wide range of genomic technologies are available to help address questions of interest to evolutionary biologists and ecologists. Microarrays are such a technology (Slonim, 2002) and provide a platform from which scientists can relate phenotypic variation in physiological traits to underlying genes and gene networks. Few groups, however, have tapped the full potential of gene expression profiles especially as they relate to understanding how genetic change translates to phenotypic variation and the resultant arrival of adaptive physiological traits.

David Weston, a postdoctoral scientist at Oak Ridge National Laboratory and colleagues are tackling this challenge by coupling microarrays with two analytical advances from the biomedical community (Horvath *et al*., 2006; Lamb *et al*., 2006). In a multistep procedure, microarrays are used to determine up- and down-regulated genes for plants exposed to an environmental stress (Step 1). The physiological state of that individual is assigned by scanning the unknown stress phenotype against a compendium of phenotypes that includes reference expression signatures derived from plants exposed to drought, heat, osmotic, salt, UV-B, and cold stress (Step 2; Kilian *et al*., 2007). A novel weighted gene coexpression network approach (Horvath *et al*., 2006) is then used to determine signaling networks and core hub genes underlying the expression phenotype (Step 3). Integration of genomic signature information with network properties is accomplished to create a more complex and informative genomic signature (Step 4). In the final step, network properties are linked with genetic information and the approach is applied in various areas of biological research (Step 5).

Steps involved in creating a genomic signature using gene expression data from microarrays.



Weston and colleagues argue that such an approach, if successful and further verified, will allow the plant biologist to classify the stress phenotype of an individual organism and then link that information to the underlying signaling pathways and genes that govern the response. Particularly promising is the potential to link complex genomic signatures with genetic information, thereby providing a means to use this functional genomics approach within a population genetics context (i.e. ecological genomics).

Although early in the development of these ideas, Weston indicates that this signature-based approach coupled with network analysis can be used to interrogate the fundamental underpinnings of complex biological systems. It is likely that this concept will find applications in quantitative genetics, comparative physiology, and population biology. Thus, genomic signatures, as a complement to traditional microarray analysis, could serve as a new tool for scientific discovery.

advanced mapping populations and developing techniques to facilitate rapid and nondestructive phenotyping of individuals in large numbers. Access to these resources has made it easier to find genes underlying traits of interest by means of quantitative trait loci (QTL) mapping and through association studies in natural populations (Weinig *et al.*, 2003; Stinchcombe & Hoekstra, 2007). Such approaches are now widely used by evolutionary biologists to investigate the genetic basis of drought avoidance strategies in desert annual sunflowers (Donovan *et al.*, 2007); the production of flavanoids thought to influence the resistance of plants to ultraviolet radiation (de Meaux *et al.*, 2006); and the response of flowering time to latitude in Arabidopsis (Stinchcombe *et al.*, 2004). An outstanding illustration of how genes contribute to adaptive traits was highlighted in the presentation by Hopi Hoekstra (Harvard University, MA, USA). She and colleagues recently conducted an association study to assess the contribution of the melanocortin-1 receptor gene (*MC1R*) to pigmentation differences between light-colored beach mice that inhabit Gulf Coast barrier islands and dark-colored con-specifics from the mainland (Hoekstra *et al.*, 2006; Steiner *et al.*, 2007). A single amino acid mutation in *MC1R* explained up to 35% of the variance in seven different pigmentation phenotypes. This study demonstrated that single nucleotide changes could have large effects on quantitative traits and that alterations at the scale of the genome could have potential consequences for how organisms interact at landscape scales. Likewise, Thomas Mitchell-Olds (Duke University, NC, USA) showed results for *Boechera stricta*, a close relative of Arabidopsis, where a QTL controlling resistance to insect herbivory had been mapped to a small chromosomal region. He reported that positional cloning efforts were underway to identify this locus, and that near-isogenic lines had been developed for the trait of interest. Access to such near-isogenic lines will enable analyses of the ecological and fitness consequences of this important gene within and among natural populations. The audience was intrigued with the possibility that the consequences of genetic manipulations could be tested using standard genetic approaches (i.e. plant breeding). These studies, and others presented during the symposium, while not yet fully embracing a genome-wide search for genes controlling complex adaptive traits, nonetheless move us closer to realizing the utility of functional genomics in evolutionary biology by identifying the mechanisms that underlie adaptive traits via a candidate gene approach based on *a priori* knowledge from quantitative genetics.

Climate change as a selective force driving evolution

Climate is an important factor driving plant performance and evolutionary change in natural and managed ecosystems. Specifically, climate change – rising CO_2 concentrations, increases in global temperatures and regional changes in precipitation – are creating novel environments and these changes are likely to act as strong selective agents on traits known to impact fitness. Droughts and other anticipated changes resulting from shifts in precipitation intensity and frequency may be particularly potent selective forces, especially in arid regions (Franks *et al.*, 2007). Several presentations focused on approaches that might be useful in identifying traits through which natural selection may act to alter fitness in these novel environments. One approach is to examine physiological traits that confer high fitness in closely related species growing in different environments. This approach has been used to identify adaptive traits related to nutrient and water use efficiency that confer high fitness in a desert Helianthus hybrid system (Brouillette *et al.*, 2007). A second approach is to examine traits closely related to fitness – like flowering time – and identify the extent to which such a trait varies in response to altered resource availability. Clint Springer and Joy Ward (University of Kansas, KS, USA) provide evidence in their recent *New Phytologist* Tansley Review that significant intraspecific variation exists in the response of flowering time to increased atmospheric CO_2 (Springer & Ward, 2007). Such variation suggests a high potential for flowering time to undergo natural selection and thus influence evolutionary processes. This finding is further supported by evidence from other experiments that also pinpoint flowering time as a key trait in increasing the fitness of plants selected for high seed number at elevated CO_2 (Ward *et al.*, 2000; C. J. Springer & J. K. Ward, unpublished). Once these traits have been identified, the underlying mechanisms can be examined using functional genomics. Johanna Schmitt (Brown University, Providence, RI, USA) reported results from a large common garden experiment designed to address how natural variation in candidate flowering time genes affect phenotype and fitness for over 300 ecotypes of Arabidopsis. She and colleagues had observed that several candidate flowering genes, in particular *FRIGIDA*, were associated with life history variation and relative fitness, but that these effects differed among sites and seasonal cohorts. Identification of traits that contribute to high fitness, and knowledge regarding the underlying genetic mechanisms responsible for natural variation in these traits, will dramatically increase our ability to scale these findings across a broad range of species and ecosystems.

Scaling from genes to ecosystems

The structure and function of terrestrial ecosystems can, in simple terms, be described by the response of individual organisms to multiple environmental and genetic factors and by the integration of those responses across multiple levels of biological organization. Significant progress has

been made in understanding how biotic and abiotic factors influence biological systems at discrete levels of organization (e.g. cellular, organismal, ecosystem). There are, however, gaps in our understanding of the biological integration that underlies how ecosystems change in response to the environment. Techniques to model metabolic networks (Sweetlove & Fernie, 2005) and an ever-widening array of bioinformatics tools are available for integration into ecological research in an effort to bridge these knowledge gaps. Genome-wide patterns of gene expression, and high-throughput enzyme and metabolite profiling (Hall, 2006), are beginning to reveal the mechanisms that underlie changes in plant phenotype and ecology under stress conditions and scenarios of climate change. For instance, the use of these tools is starting to reveal the transcriptional regulation of acclimation in plants, which allows them to: (1) make use of greater photoassimilate availability at elevated CO_2 (Ainsworth *et al.*, 2006; A. D. B. Leakey *et al.* unpublished); (2) increase the efficiency of cellular ATP utilization under foliar phosphorus-deficient conditions (S. S. Thayer *et al.* unpublished); and (3) shift resource allocation towards heat-shock proteins, which can limit damage during drought stress (Watkinson *et al.*, 2003). The mechanistic basis for genotypic variation in plant responses to the environment has long been enigmatic, and genotype-specific patterns of gene expression provide a uniquely integrated view of the anabolic, catabolic and signaling processes that may combine to regulate whole plant performance.

Reverse genetics is also being used to characterize how manipulating gene function translates to consequences at the level of populations and ecosystems. Scientists at Brookhaven National Laboratory, for example, are working to characterize how partial deletion of the nitrate reductase gene scales from enzymes to ecosystems in simplified Arabidopsis mesocosms. Alistair Rogers and colleagues measured gene expression, enzyme activities and metabolite pools on wild-type plants and in *nia2* mutants (compliments of Nigel Crawford, University of California, San Diego, CA, USA), and showed that shifts in carbon and nitrogen metabolism owing to a reduced nitrate reductase activity delayed the flowering time, reduced the reproductive biomass and negatively impacted seed germination. These effects tipped the competitive balance in favor of the wild-type plants and over multiple generations a decline in the relative abundance of mutants in mixed mesocosms was observed.

Although the majority of presentations focused on scaling plant-related processes, extrapolation from organismal-scale responses to ecosystem function must also consider functional and compositional shifts in microbial communities and the complex interaction of plants and microbes as they relate to changes in soil nutrient availabilities. Eoin Brodie (Lawrence Berkeley National Laboratory, CA, USA) presented results from an annual grassland mesocosm experiment which indicated that variation in soil water content could lead to shifts in microbial community structure. Sarah Placella (University of California, Berkeley, CA, USA) then showed that differences in the nitrifier community explained 45% of the variation in nitrification potential, a measure of microbial activity that is a strong indicator of plant available nitrogen. Subsequent analyses, in collaboration with Stephanie Bernard (Lawrence Berkeley National Laboratory, CA, USA), showed that the abundance of transcripts for one gene encoding nitrate reductase, which is involved in nitrate assimilation in plant leaves, was strongly correlated with nitrification potential. Although the foci of these talks were the strong linkages between microbial nitrogen transformations and plant nitrogen processing, the role of microbial composition on other ecosystem processes was also emphasized, including soil respiration and carbon dynamics.

Conclusions

The co-organizers of these two symposia had a common goal, namely to facilitate communication between ecologists, evolutionary biologists and researchers with expertise in functional genomics. This was carried out with the purpose of understanding in greater detail how genes help to shape the structure and function of terrestrial ecosystems, and how those insights can help us to predict the response of plants and animals to biotic and abiotic stresses in a changing world. High-throughput tools now exist for collecting data on thousands of genes for organisms living in their natural environments, and, as we heard, ecologists and evolutionary biologists are beginning to use these tools in imaginative ways. There are, unfortunately, as humorously pointed out by Hopi Hoekstra (Harvard University, MA, USA), no plans for high-throughput ecology. We can design laboratory and field experiments to accelerate some ecological processes and thus condense the time required to observe critical connections among genotype, phenotype and ecosystem function, but even these experiments require time. This alone, however, should not hinder the integration of functional genomics into ecology or evolutionary biology. We should recognize that as we move forward, whether that be in small steps or giant leaps, the challenges likely to be encountered are many, as too are the rewards.

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Key words: adaptive traits, climate change, ecology, evolution, functional genomics, gene expression, microarrays.

The many faces of climate warming

Ecosystem responses to experimental warming and other global climate change factors: Organized session at the Ecological Society of America (ESA) 92nd Annual Meeting, San Jose, CA, USA, August 2007

The release of the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC) this past February raised the stakes on role of climate warming in our planet's future. Within the next century our climate is likely to warm by 1.1–6.4°C in concert with rising concentrations of greenhouse gases, largely reflecting human influences on radiative forcing (IPCC, 2007). The prospect of climate warming coupled with elevated atmospheric concentrations of carbon dioxide, altered precipitation patterns, and increased nitrogen deposition presents a tangled array of global change drivers and the

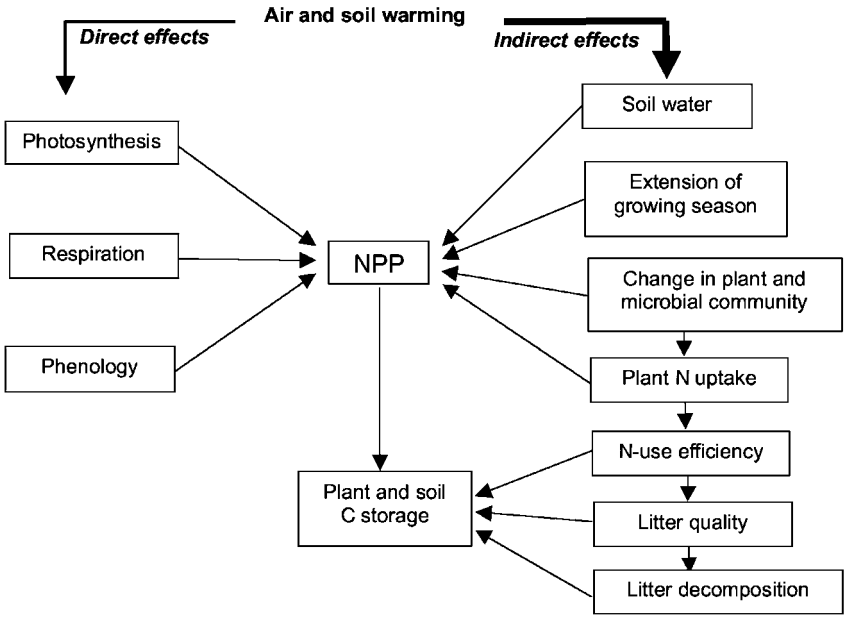

Fig. 1 The major factors controlling the response of net primary production (NPP) and carbon (C) storage to the effects of climate warming in terrestrial ecosystems.

potential for complex effects on the structure and function of terrestrial ecosystems.

Over the past decade, much progress has been made in experimental research and long-term observational studies quantifying the nature and magnitude of climate-warming effects on terrestrial ecosystems and linking them to coupled atmosphere-biosphere processes. Disentangling the direct and indirect effects of warming on ecosystems remains a key conceptual and experimental challenge. To this end, multi-factorial experiments and modeling efforts will be key to developing science-based predictions of ecosystem responses to warming (Norby & Luo, 2004). A session organized by Xuhui Zhou and Yiqi Luo (University of Oklahoma, OK, USA) at the 92nd Annual Meeting of the Ecological Society of America in San Jose, CA, USA, in August was aimed at summarizing the findings to date concerning the multiple roles of climate warming in a variety of ecosystems.

'... in the coterminous United States, the frost-free period has extended by as much as 25 d over the past 50 yr'

How important are the direct effects of temperature?

Although temperature affects many terrestrial ecosystem processes, one of the most striking observations to date and prima facie evidence of climate-warming effects has been the extension of the growing season in various climatic zones. For example, in the coterminous United States, the frost-free period has extended by as much as 25 d over the past 50 yr. As Christopher Field (Carnegie Institution of Washington, Stanford, CA, USA) noted at the outset of the morning session, warmer temperatures coupled with a longer growing season should increase net primary production (NPP) as climate warming may directly enhance photosynthetic carbon assimilation (Fig. 1). Indeed, increased NPP in response to warming is observed in a number of experiments, but not always (Rustad *et al.*, 2001; Dukes *et al.*, 2005).

Further studies should enable a critical test of whether or not warming effects on NPP are, as a rule, greater in cooler than in warmer climates, where temperature limitations on productivity are thought to be larger. In warmer climates limitations imposed by water balance may constrain NPP responses to warming and amplify the indirect effects of increased temperature on reducing soil water content via increased evapotranspiration (Fig. 1). Indeed, in tallgrass prairie exposed to climate warming in combination with altered precipitation distribution, reported by John Blair and colleagues (Kansas State University, Manhattan, KS, USA), warming reduced above-ground NPP and soil CO_2 efflux, supporting the notion of water deficit-mediated responses to climate warming.

Given the fundamental nature of the relationships between temperature and plant metabolism, predicting direct temperature effects on photosynthesis and respiration might seem straightforward. Yet we have long known that temperature acclimation modulates the direct effects of temperature on carbon exchange rates in plants (Atkin & Tjoelker, 2003). Recent studies suggest that temperature acclimation may also be an important modulator at the ecosystem scale in

terms of respiratory CO_2 efflux from plants and soils (Luo *et al.*, 2001), mitigating direct temperature effects on changes in carbon pools and fluxes (King *et al.*, 2006), and rendering simple simulations based on first principles (i.e. Q_{10} or Arrhenius functions) problematic at best.

To be sure, experimental climate warming often results in increased respiratory carbon losses, particularly from soil organic carbon pools. At Harvard forest in Massachusetts, USA, and Flakaliden, Sweden, decade-long soil-warming experiments revealed increased CO_2 fluxes from soil to the atmosphere. However, the responses were small and transient or diminished through time (Melillo *et al.*, 2002; Eliasson *et al.*, 2005), likely owing to limited pools of labile soil carbon and perhaps reflecting constraints ultimately set by photosynthetic carbon assimilation. Likewise, Richard Gill (Washington State University, Pullman, WA, USA) reported transient and nonsignificant soil respiratory responses of a subalpine meadow to experimental warming. Sorting out the relative contributions of autotrophic and heterotrophic respiration and soil carbon pool dynamics will continue to be an important research objective in warming studies.

The emergence of indirect effects

The indirect effects of global warming on terrestrial ecosystems are likely more important than direct effects (Shaver *et al.*, 2000; Luo, 2007). This was a recurring theme throughout the session. Climate warming influences ecosystem processes by extending the length of the growing season and changing plant phenology (Harte & Shaw, 1995; Wan *et al.*, 2005), increasing soil nitrogen (N) mineralization and availability (Rustad *et al.*, 2001), reducing soil water content (Wan *et al.*, 2005), and shifting species composition and community structure (Shaver *et al.*, 2000; Wan *et al.*, 2005). Warming-induced changes in soil N transformations can trigger long-term feedbacks on ecosystem carbon balances because N strongly regulates terrestrial carbon sequestration, potentially enhancing carbon storage. Warming and associated drought may stimulate below-ground growth, increase root/shoot ratios, and result in shifts of the plant community to C_4 species, shrubs, and other drought-tolerant species. The experimental evidence on indirect effects and interactive effects of warming certainly provides a challenge to modeling efforts of global carbon-climate feedbacks, moving beyond the kinetics of photosynthesis and respiration (Luo, 2007).

The future faces of warming: state changes in ecosystems

Determining the nature and tempo of successional changes in ecosystems in response to warming remains a key challenge. Changes in ecosystem states through altered species composition and dominance will have profound effects on NPP and biogeochemical cycles, perhaps surpassing the direct effects of global change drivers themselves. In particular, feedbacks between plant functional types and soil processes, including effects on microbial communities, are poorly understood in this context.

Will climate warming and other global change drivers promote certain species or plant functional groups over others? Early experiments by Harte & Shaw (1995) demonstrated warming-induced shifts in species dominance in favor of perennial woody shrubs in a montane meadow ecosystem. Margaret Torn and colleagues (Lawrence Berkeley Laboratory, Berkeley, CA, USA), in a study of California annual grassland, reported altered productivity and species abundances in response to warming and interactive effects with precipitation amount. In an old field community in Tennessee, Amiee Classen and colleagues (Oak Ridge National Laboratory, Oak Ridge, TN, USA) reported complex responses of plant functional groups to the combined effects of warming, elevated CO_2, and water availability, including differences among tree species in seedling establishment. In oak savanna in central Texas, the work of one of us (MGT) suggests that encroachment of invasive *Juniperus virginiana* may increase in future, warmer climates. Further surprises are likely in store, owing to constraints on intraspecific plant adaptation and range shifts in fragmented landscapes (Davis & Shaw, 2001). To date, few if any studies have experimentally tackled these landscape-scale questions in an ecosystem framework.

The future of experimental warming studies

A variety of approaches to experimental warming are available, each with advantages and limitations. Glasshouse mesocosms, open-top field chambers, infrared warming, passive nighttime warming, and soil warming are among the techniques, many of which were reported on in the 3 h session. Yet surprisingly, we know relatively little about forest ecosystem responses to experimental warming. Unlike free-air CO_2 enrichment (FACE) studies, which have approached their golden age, spanning diverse vegetation types in nearly every continent, field-based warming studies to date remain largely restricted to small plots and comparatively short-statured vegetation. The development of methods to warm both the air and soil of large-scale forest plots will be an important technical advance.

Ecosystems across the globe have already been exposed to increased temperatures for almost two decades. Long-term observational data will no doubt contribute further insight into warming effects. Yet many gaps remain in our knowledge of the impacts of global warming on ecosystem processes. For example, long-term observations and model simulations show that daily minimum temperatures have increased at a faster rate than daily maximum temperatures (Easterling *et al.*, 1997). Shuli Niu (Chinese Academy of Sciences, Beijing, China) demonstrated differential effects of day vs

nighttime warming in a temperate steppe in China, showing increased carbon uptake in response to night warming compared with day warming and control treatments. In a grassland in Oregon, Jillian Gregg (Terrestrial Ecosystems Associates, Corvallis, OR, USA) is testing whether increased carbon assimilation with warmer mornings will offset the greater respiratory costs with warmer night temperatures. These studies underscore the continuing need to resolve ecosystem responses in terms of underlying photosynthetic and respiratory physiology.

How other ecosystems, such as forests, savanna, and deserts, will respond to the many faces of warming is largely unknown. In the meantime, synthesis and modeling activities remain important tools. Nonetheless, the scientific community appears poised to address these questions in an integrative manner. Given the prospects of rapid climate warming, science-based predictions of ecosystem responses will certainly play an important role in the policy debates concerning adaptation and mitigation strategies.

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Meta-analysis: the past, present and future

Synthesizing ecological studies in a changing world using meta-analysis: Organized session at the Ecological Society of America (ESA) 92nd Annual Meeting, San Jose, California, USA, August 2007

The use of meta-analysis in the field of ecology has increased exponentially since its introduction in the early 1990s. Meta-analysis is a set of statistical techniques that enables researchers to combine the results from a number of independent studies. Meta-analysis is therefore the analysis of analyses, as implied by the name. The techniques for ecological meta-analysis have been borrowed from other disciplines, primarily the medical, physical and behavioral sciences (Gurevitch & Hedges, 1999). These techniques have also been adapted for ecology, and new metrics have been developed specifically for ecological questions (e.g. response ratio; Curtis & Wang, 1998; Hedges *et al.*, 1999). Furthermore, the development of easy-to-use statistical software (e.g. METAWIN, Rosenberg *et al.*, 2000) has rapidly expanded the use of meta-analyses in ecology. An organized oral session (OOS) at the 2007 Ecological Society of America (ESA) meeting focused on the historical evolution of meta-analyses in ecology, the current use in synthesizing results from global change studies and the future of meta-analyses in ecology. In this article, we present some highlights and future challenges proposed in the session.

'Since the early 1990s, there have been over 700 published meta-analyses in ecology and evolution'

A brief history

While meta-analyses have been used for several decades in other disciplines, their use in ecology did not really take off until the 1990s. In their seminal synthesis of field experiments of competition, Jessica Gurevitch (Stony Brook University, NY, USA) and colleagues laid the groundwork for using meta-analysis for ecological data (Gurevitch *et al.*, 1992). They suggested that meta-analyses could fundamentally alter the way that ecologists draw conclusions from the outcomes of experiments. Specifically they suggested that meta-analyses could lessen the focus on so-called 'textbook examples' and instead adjust the focus to the quantitative synthesis of separate, independent studies. Furthermore, meta-analyses allow an alternative approach to traditional, narrative reviews or statistically flawed quantitative approaches such as 'vote-counting' reviews. Meta-analyses offer a number of important advantages, including the ability to calculate effect size estimates (i.e. the overall magnitude of responses) and to discriminate statistically among the effect in different subsets of studies. A goal and an inherent part of the philosophy underlying meta-analysis is that it requires the same rigor in sampling and analysis as is required in primary research. The application and influence of meta-analysis in ecology has continued to expand in recent years. Since the early 1990s, there have been over 700 published meta-analyses in ecology and evolution (reported by Gurevitch and Julia Koricheva, University of London, UK).

What ecological questions has meta-analysis addressed?

The area in which meta-analysis has had the greatest impact is perhaps global environmental change, particularly in the effects of elevated CO_2 on plant physiology and growth. Meta-analysis was first used to synthesize the results from elevated CO_2 studies on gas exchange variables and leaf nitrogen (N) by Peter Curtis (Ohio State University, OH, USA; Curtis, 1996). The earlier CO_2 meta-analyses, although focused primarily on studies with relatively short experimental durations, provided statistical confirmation of a number of key responses to elevated CO_2 in trees (Curtis, 1996; Curtis & Wang, 1998). More importantly, the work by Curtis and colleagues highlighted the areas of uncertainty in our understanding of the plant response to elevated CO_2 and, in doing so, has had a large influence on subsequent primary research and has changed the complexion of CO_2 study as an ecological subdiscipline. Over the last decade, approximately 50 papers using meta-analytical techniques have been published to synthesize results of the large number of ecological CO_2 studies that have been conducted.

One important feature of meta-analysis that is lacking in empirical studies or traditional reviews is its ability to synthesize results from independent studies in a manner that is both objective and statistically defensible. This feature makes meta-analysis a powerful tool and has revised some earlier assumptions and findings in ecology. For example, it was hypothesized that plant species with the C_4 photosynthetic pathway would have a lower responsiveness to elevated CO_2 and therefore could lose the competitive advantage to C_3 species as the CO_2 level in the atmosphere continues to rise. Meta-analyses by Wand *et al.* (1999) and Poorter & Navas (2003), however, found a significant increase in the growth of C_4 species at elevated CO_2 and thus called for a critical re-evaluation of the assumption of lower growth responsiveness in C_4 species to elevated CO_2. In a recent analysis of production of crops grown under free-air CO_2 enrichment (FACE) conditions, Ainsworth & Long (2005) found that crop yields increased far less than anticipated from previous enclosure studies. The important quantitative difference detected by this meta-analytical synthesis (Ainsworth & Long, 2005), as well as the finding of lower levels of proteins and essential minerals in staple crops grown at elevated CO_2, based on a meta-analysis by Daniel Taub and colleagues (South-western University, TX, USA), will have significant implications for food production and human nutrition in the future.

One trend of CO_2 meta-analysis on plant physiology and growth seems to be the synthesis of studies on multiple environmental changes, particularly elevated O_3 (discussed by Elizabeth Ainsworth, University of Illinois, IL, USA). A comprehensive analysis of the publications of O_3, alone or in combination with elevated CO_2, for example, demonstrated significant interactive effects of O_3 and CO_2 on leaf chemistry and some indices of insect performance (Valkama *et al.*, 2007). Another trend in CO_2 meta-analysis is to elucidate mechanisms governing plant responses to elevated CO_2. In a recent synthesis of 411 CO_2 publications, Wang (2007) found that plant assemblages of single species (population) were more responsive to elevated CO_2 than assemblages of multiple species (communities) in biomass accumulation. The meta-analytical findings led to the formulation of the resource usurpation hypothesis (i.e. competitive compartmentation of growth-limiting resources by less responsive plant species), which may be important in determining the growth response to elevated CO_2 in a community (Wang, 2007).

In addition to synthesizing studies of elevated CO_2 on plant physiology and biomass accumulation, meta-analysis has been used to examine CO_2 effects on plant characteristics that can affect C and nutrient cycling. Nitrogen concentration, for instance, showed a small but consistent decline, whereas leaf lignin increased by 6.5% in leaf litter from elevated CO_2-grown plants (Norby *et al.*, 2001). It was thus concluded

that litter decomposition would be slower in a higher CO_2 environment. A more recent synthesis of 104 publications demonstrated that elevated CO_2 stimulated net accumulations of C and N in terrestrial ecosystems, which may help to prevent the complete down-regulation of long-term CO_2 enhancement of C sequestration (Luo *et al.*, 2006).

The CO_2 responses of organisms other than plants have also been examined using meta-analytical techniques. The effects of environmental changes on the responses of soil organisms and mycorrhizas have significant implications for global C and nutrient cycling. Soil organisms of different trophic levels (detritivores and herbivores at the second trophic level, bacterivores and fungivores at the third level and predators at the fourth level) have been found to vary in their responses to environmental changes, including higher CO_2 (discussed by Joey Blankinship, Pascal Niklaus and Bruce Hungate, Northern Arizona University, AZ, USA and Swiss Federal Institute of Technology, Zurich, Switzerland). Results from an earlier meta-analysis demonstrated that mycorrhizal abundance decreased with the addition of N and phosphorus (P), but increased by 47% at an elevated level of atmospheric CO_2 (Treseder, 2004). These meta-analytical studies were able to statistically generalize results from a large number of individual studies to reach basic and applied conclusions, e.g., support of the plant investment hypothesis (Treseder, 2004).

The scope of meta-analysis in synthesizing ecological studies on global environmental changes is being expanded beyond CO_2 studies. Analysis of the vegetation response to N addition found that biomass growth and tissue N concentration was affected by multiple factors, including precipitation and latitude (discussed by Shuli Niu, Shiqiang Wan and Jianyang Xia, Institute of Botany, Academia Sinica, China). A recent meta-analysis on the responses of plant communities to experimental warming indicated that warming would have negative effects on tundra biodiversity, which will have far-reaching implications for the functioning of ecologically important tundra systems (Walker *et al.*, 2006). There are a number of other global change areas (e.g. habitat fragmentation, urbanization and spreading of non-native species) that have successfully used meta-analysis to synthesize statistically the ever-increasing number of independent studies (discussed by Jessica Gurevitch, Stony Brook University). These meta-analytical syntheses have made significant contributions to the advancement of ecology as a science.

Challenges ahead

As meta-analysis has now begun to gain widespread acceptance in ecology, we face the challenge of making sure that meta-analysis is used correctly and to its full potential. This includes the use of better statistical methods as well as the proper formulation of questions that can be answered through meta-analysis.

Statistically, most ecological meta-analyses have a long way to go before they approach the sophistication of meta-analyses in other disciplines, such as medicine. At present, many ecological meta-analyses consist of sets of contrasts, functionally equivalent to performing multiple sets of single classification analysis of variance (ANOVA) tests. More advanced statistical approaches (e.g. two-way ANOVA, analysis of covariance (ANCOVA), regression, and multivariate analysis) are rarely undertaken in ecological meta-analyses. It is as if ecological meta-analysts have become stuck halfway through a standard biostatistics text, but are unable to read the rest of the book. Some specific challenges for ecologists highlighted in the session (discussed by both Michael Rosenberg, Arizona State University, and Jessica Gurevitch, Stony Brook University) include the use of hierarchical nested analyses, accounting for the effect of phylogenetic relationships within the data, and the use of advanced statistical inference methods, such as maximum likelihood and Bayesian meta-analysis. A demonstration of the power of the Bayesian meta-analysis approach was presented by Kiona Ogle (University of Wyoming, WY, USA).

As global change ecologists rise to these challenges, it is believed that meta-analysis will become an increasingly indispensable tool in ecological studies. The overall response to environmental changes produced by the meta-analytical synthesis of individual studies will not only improve our understanding of ecosystem functioning in a changing world, but also provide the information necessary to proactively plan for the future.

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Key words: carbon cycling, elevated CO_2, elevated O_3, global changes, meta-analysis, nutrient cycling, terrestrial ecosystems.

Mycorrhizas take root at the Ecological Society of America

Mycorrhizal ecology and related sessions at the Ecological Society of America (ESA) 92nd Annual Meeting, San Jose, CA, USA, August 2007

Mycorrhizal symbioses play an important role in virtually all terrestrial ecosystems (Smith & Read, 1997). They are known to have significant impacts on carbon and nutrient cycling, soil formation and structure, plant productivity and diversity, and food web dynamics (Van der Heijden & Sanders, 2002). Although the importance of mycorrhizas is widely recognized, the study of these symbioses has historically been divided between two groups of scientists. Ecologists interested in this topic have mainly focused on the above-ground part of the symbiosis (i.e. the plants) and treated the below-ground part of it (i.e. the fungi) largely as a 'black box'. In contrast, mycologists have primarily focused on the fungi themselves and given less attention to the way in which these symbioses affect plants and other organisms. Despite their common interest, a look at the early mycorrhizal literature would indicate that ecologists and mycologists rarely interacted with each other. The division between these two groups, however, appears to be quickly disappearing. This was most recently evidenced at this year's Ecological Society of America (ESA) meeting in San Jose, CA, USA, where a record amount of research on mycorrhizal symbioses was presented. Four oral sessions and a poster session were devoted entirely to mycorrhizal ecology. More significantly, research involving the symbiosis was included in 23 different general sessions and made appearances in many talks devoted to other topics. The meeting was also the first gathering for the Fungal Environmental Sampling and Informatics Network (FESIN: http://www.bio.utk.edu/fesin/), which will have alternating meetings over the next 4 yr between ESA and the Mycological Society of America in order to bring these two groups of scientists closer together. Here we summarize a few of the highlights of the mycorrhizal work that was reported at the meeting.

'... researchers are increasingly finding new and innovative ways to test questions about mycorrhizal fungi under ecologically realistic conditions.'

Molecular techniques

One fundamental aspect of ecological studies is the ability to identify the number of species present in a given area or sample. Because the active part of the mycorrhizal symbiosis occurs below-ground, researchers have increasingly relied on molecular techniques to assess the number of fungal species in their studies (Horton & Bruns, 2001). While the methods themselves have typically held center stage in research on mycorrhizal assemblages, this year's meeting showed that they have largely become second nature and the focus has shifted to how these techniques can be applied to

address broader ecological questions. For example, Shannon Schechter (University of California, Berkeley, USA) used a cloning and sequencing approach to compare the arbuscular mycorrhizal (AM) fungi associated with serpentine and nonserpentine ecotypes of *Collinsia sparsiflora* growing in adjacent plots. She found major differences in AM assemblage structure between the two ecotypes, suggesting that AM fungi may play a key role in plant adaptation to extreme soil environments. Jeri Parrent (Swedish Agricultural University, Sweden) examined the ectomycorrhizal (EM) root tip and hyphal assemblages in the Free Air Carbon Enrichment (FACE) plots in Duke forest, NC, USA and showed that increased carbon dioxide concentrations significantly shifted EM assemblage composition (Parrent *et al.*, 2006). Interestingly, many of the dominant fungi had different patterns of root tip to hyphal ratios between treatments, indicating that the assemblage shift may have important functional consequences in host plant response to global change. Working in three temperate hardwood forests in north-eastern Michigan, Ivan Edwards (University of Michigan, USA) found a strong spatial stratification of saprotrophic and EM fungi in soil litter and mineral soil horizons and showed that EM mycelium dominated the soil even in an *Acer* / *Tilia* forest where *A. saccharum*, the main dominant tree, is an AM associate. These studies, as well as many others presented at the meeting, demonstrate that the doors initially unlocked by molecular techniques are now open, providing significant insight into the ecological role of this symbiosis.

While molecular identification techniques have allowed mycorrhizal researchers to ask more ecologically based research questions, they have also revealed the high species richness of mycorrhizal assemblages (Dahlberg, 2001). The richness of these assemblages is often on the same order as that of the richest plant and animal assemblages and can be particularly high in areas with multiple host species (Ishida *et al.*, 2007). Our perceptions of mycorrhizal species richness are, however, strongly affected by sampling strategy (Horton & Bruns, 2001; Taylor, 2002). Lee Taylor (University of Alaska, Fairbanks, USA) provided another example of the domination of EM fungal mycelium in forest soil; this pattern has now been seen across an impressive array of northern temperate forest types (O'Brien *et al.*, 2005; Lindahl *et al.*, 2007; Ivan Edwards, this meeting). In addition, he showed that sampling fungal assemblages to saturation (i.e. capturing all of the species present in a given sample) can be challenging even in forests dominated by a single EM host species. Using a cloning and sequencing approach, he compared the diversity of fungal taxa found in 99 *Picea mariana* litter samples (380 clones) with that of one *P. mariana* litter sample (1080 clones). He found that, in both cases, the species–effort curves had still not plateaued and that approximately 50 fungal taxa were present in a 0.25-g soil sample! Interestingly, even some of the most common sequence types within the single sample were not recovered again in the pooled sample that included it. This inability to saturate fungal collecting curves has been previously seen in root-tip surveys (reviewed in Horton & Bruns, 2001) and in a clone pool study of Duke forest soil (O'Brien *et al.*, 2005). In fact, the only cases in which saturation has been clearly achieved are those where only EM fungi were targeted, the forest was young, and the spatial scale was relatively small (Horton & Bruns, 2001; Peay *et al.*, 2007).

Top-down vs bottom-up trophic interactions

Another factor bringing research on mycorrhizal fungi into the ecological mainstream is their integration into topics that have long interested ecologists. One of the classic debates in ecology has been about the relative importance of top-down vs bottom-up control of trophic interactions (see special feature articles in *Ecology* 73(3)). Although a sizable literature has developed on this topic, the role of mycorrhizal fungi has largely been overlooked. Working in Canadian grassland, J. C. Cahill (University of Alberta, Canada) examined the effects of AM fungi on plant–pollinator interactions. He found a strong bottom-up effect on pollinator community composition, with a 67% reduction in bumble bee visits in plots where mycorrhizal fungi were removed with benomyl. Conversely, in a semiarid woodland, Kitty Gehring (Northern Arizona University, USA) showed that parasitism (from mistletoe), competition (from other trees), and herbivory (from scale insects) on plants all have major independent top-down effects on EM assemblage structure. Interestingly, the EM assemblages of stressed plants converged on a core group of Pezizalean ascomycetes. Working in the same system, Chris Sthultze (Northern Arizona University, USA) followed up on the differences in EM assemblages between pines that are resistant or susceptible to the pitch mass borer, *Dioryctria ponderosae*, and showed that, even if the insect was experimentally removed for many years, susceptible and resistant trees continued to show distinct assemblages. Even more surprisingly, he showed the same pattern for seedlings of resistant and susceptible trees, which indicated that the differences were driven at least in part by host genetics. Studies such as these clearly indicate that mycorrhizal fungi both affect and are affected by organisms at other trophic levels. Like the classic lynx–hare dynamics that are now realized to be influenced by additional trophic interactions (Krebs *et al.*, 1995), the increased inclusion of mycorrhizal fungi in ecological studies will clearly lead to a fuller understanding of the factors that control multitrophic dynamics.

Models and manipulative experiments

Mycorrhizal researchers are also increasingly interested in using tools developed by ecologists and testing ecological

theories developed for other organisms on fungi. Modeling, for example, has long been used in ecology to generate predictions that can then be tested with empirical studies. Miroslav Kimmel (Colorado College, USA) presented a set of biological market models examining the optimal number of fungal symbionts with which a plant should associate (Kimmel & Salant, 2006). He found that the shape of the carbon–nitrogen trading curve (i.e. whether it is concave or convex) is likely to play a determining role in whether a plant will associate with one or multiple fungi. Many talks also showed the increased use of manipulative experiments to test the types of questions generated by models or correlation-based studies. Using a split-root experimental design, Jim Bever (Indiana University, USA) demonstrated that plants are able to differentially reward fungal symbionts that provide the most nutrients, but this reward system is apparently effective only in spatially structured environments. In a uniform pot setting, negative feedback dominates; that is, the worst fungal symbiont from the plant's perspective has a competitive advantage. This may be attributable to difference in scale between the fungal and plant partners. If a plant is not actually rewarding the best fungus directly, but rather is investing more in the most nutrient-rich part of its root system, then, when different fungi are intermingled at fine scales, the plant may not be able to direct rewards to the best symbiont and the fungus that provides little may reap a disproportionate reward for its limited effort.

Competition among mycorrhizal fungi was also the focus of P. Kennedy's talk (Lewis and Clark College, CA, USA). Manipulative laboratory and field experiments on a set of four EM fungi in the genus *Rhizopogon* showed that, when these fungi compete for colonization of pine seedling roots, timing, host root density, and inoculum type all matter. Differences in the speed of spore germination had been previously demonstrated to be a major determinant in competition between two of these *Rhizopogon* species (Kennedy & Bruns, 2005). Such 'priority effects' are well known in other organisms (Keddy, 2001) and are based on one competitor capturing a resource and making it unavailable to a second. New experiments showed that this was a general property among three of the *Rhizopogon* species, and that when order of colonization was artificially manipulated outcomes could be reversed. Although there are still significant challenges to manipulating mycorrhizal assemblages in the same way as those of other organisms, particularly in field settings, researchers are increasingly finding new and innovative ways to test questions about mycorrhizal fungi under ecologically realistic conditions (see Nara (2006) for an interesting lab-field hybrid example).

Future advances

The most interesting advance in methods reported at the meeting was the use of 'quantum dots' by Matthew Whiteside and Kathleen Tresender (University of California, Irvine, USA) to examine fungal uptake and transport of organic nitrogen sources. Selected amino acids or chitosan were bound to florescent nanoparticles and used to show that AM fungi were capable of uptake and transport of both compounds. The visual images of these particles taken with florescent microscopy were striking, and it was clear from the talk that this method is easily adapted to a wide range of organic compounds. On the basis of these early results, it seems very likely that ecologists will be hearing much more about this method in the near future.

The incorporation of mycorrhizas into the general science of ecology is part of a larger trend toward the melding of the ecology of macro- and microorganisms. This trend has been spurred on by researchers in many other fields (e.g. a symposium on this topic organized by Brendan Bohannan (University of Oregon, USA) at the 2006 ESA meeting) and is evidenced by special issues of *Ecology* that focused on microbes (88(6)) and tropical fungi (88(3)). This linkage of macro- and microecology is long overdue and is destined to expand in many new and unexpected ways. Mycorrhizal fungi have provided an important bridge between mycologists and ecologists, but they are just one example of the many pervasive interactions between plants and fungi and there is much room for expansion into pathogenic, commensualistic, and saprobic interactions as well.

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