The adaptive value of leaf colour

Origin and evolution of autumn colours, Oxford, UK, March 2008

Why do leaves turn red? (Fig. 1) Such striking foliar colours seem to mean something, but what? Many temperate species develop red foliage in autumn. Young flush leaves in tropical forests are also often red. Red is the colour of the lower leaf surfaces of many understory and floating plants. Thorns and spines are often red. Stress commonly causes foliage to blush. Why does this happen?

A number of tribes of biologists have theorized about the distinctive colours of leaves, and a few researchers have even performed experiments. This has led to a range of sometimes mutually incompatible hypotheses (Archetti, 2000; Lee & Gould, 2002; Manetas, 2006). Marco Archetti (University of

Fig. 1 A wild apricot (*Prunus armeniaca*) in autumn in its native range in Kyrgyzstan (photograph by Marco Archetti).

Oxford, UK) had the idea that it was time to get some of the tribal leaders together to see if any kind of consensus could be reached. The result was a one-day conference, followed the next day by a workshop, to consider the adaptive value of leaf colour.

> *'... yellow leaves had a more advanced extension of the abscission layer through the vasculature than red and green leaves.'*

The keynote speaker was David Lee (Florida International University, Miami, USA), author of the extraordinary *Nature's Palette – The Science of Plant Color* (Lee, 2007), a book that has achieved the rare feat of breaking out of the academic ghetto into the world of newspaper reviews and popular science. He presented a (literally) colourful overview of the range of pigmentation found in plants from the orange-brown flavonoids in the cell walls of sphagnum and the red xanthophylls of the cycad *Zamia* to the betalains in the upper epidermis responsible for the bronzish appearance of young *Bougainvillaea* leaves. Most attention in the meeting was given to pigments of the anthocyanin class. There are two broad schools of thought concerning the function of the red coloration caused by anthocyanins. The plant scientists (like Lee) have suggested some variation of the photoprotection hypothesis: anthocyanins protect the leaf from the damaging effects of light at low temperatures, allowing a more efficient resorption of nutrients, especially nitrogen (Lee, 2002; Lee & Gould, 2002). The entomologists and evolutionary biologists (like Archetti) have proposed (or questioned) coevolution as the explanation: red is a warning signal of the defensive status of the tree to animals, particularly feeding insects such as aphids (Archetti, 2000; Hamilton & Brown, 2001).

Functions of autumn coloration in the physiology of the plant

Evolutionary and genetic aspects of pigment metabolism in leaf senescence were addressed in a paper by Howard Thomas and Helen Ougham (University of Aberystwyth, UK). Their review of the molecular and cell biology of chlorophylls, carotenoids and phenylpropanoids (Ougham *et al.*, 2005) raised a number of questions. Did plant–animal coevolution drive the ontogeny and evolution of the interrelationship among chloroplasts, gerontoplasts (senescing plastids) and chromoplasts? Does leaf colour signal the hazardous nature of the cell vacuole, which accumulates not only chlorophyll catabolites and autumnal anthocyanins but also aggressive hydrolases and oxidases? Has this influenced the evolution of feeding behaviour – for example grazing, or the stylet paths of sap-sucking insects? What are the resource implications of colour changes and are they significant from the fitness point of view?

Paul Schaberg (USDA Forest Service, Burlington, USA) presented results of studies on sugar maple (*Acer saccharum*). The onset and intensity of leaf reddening in this species correlate with foliar starch, sugar and nitrogen concentrations. Carbohydrate and nitrogen (N) contents are reciprocally related. Low-N/high-sugar trees turn red earlier and more completely than high-N/low-sugar individuals (Schaberg *et al.*, 2003). It is well established that there is a relationship between elevated carbohydrate status and anthocyanin accumulation (Lloyd & Zakhleniuk, 2004). The speaker showed that the redness of maple leaves could be manipulated by interfering with sugar translocation. The experiment exploited observations made over several years that identified individual trees as more or less red in autumn. Ring-girdling branches of trees that normally developed little autumnal colour resulted in elevated concentrations of both sugars and anthocyanins. Schaberg also presented anatomical evidence that red leaves may have a greater capacity for nutrient resorption than senescing yellow leaves during autumn. Microscopic analysis indicated that yellow leaves had a more advanced extension of the abscission layer through the vasculature than red and green leaves

(Schaberg *et al.*, 2008). A more limited progression of the abscission layer through vascular bundles could extend resorption of mobile leaf constituents.

The possible functions of red pigmentation in leaves of evergreens were analysed by Niky Hughes (Wake Forest University, NC, USA). She showed the range of seasonal response from spring flush to autumnal colour change, with examples from shrubs and ground-cover species (Hughes *et al.*, 2005). Three hypotheses concerning the role of red pigmentation in relation to abiotic stress were subjected to experimental test, namely low-temperature adaptation, photoprotection and osmotic adjustment. There were indications that red foliage could be somewhat warmer than green at low temperatures and low wind speeds, particularly when leaves are large. She also looked at the possibility that anthocyanins act as osmoregulatory agents, but the experimental evidence proved inconclusive. She found stronger support for a photoprotective function: measurements of photosynthetic capacity and efficiency suggested that red leaves have shade-adapted characteristics. Concentration of anthocyanins in the uppermost cell layers of mesophyll tissue in the species she studied is also consistent with a light-interception function.

Yiannis Manetas (University of Patras, Greece) pointed out that anthocyanins have absorption spectra that sit between the red and blue maxima of the chlorophyll of photosynthetic membranes and therefore are not likely to be particularly effective sunscreens. A proposed antioxidant function for anthocyanins (Neill & Gould, 2003) is critically dependent on their proximity to the source of oxy-radicals (Kytridis & Manetas, 2006); when anthocyanins and sources of oxy-radicals are in different cells or intracellular locations (e.g. chloroplasts vs the vacuole), there is little evidence for a protective effect. If anthocyanins have an adaptive function in response to abiotic stress, it should be possible to demonstrate that leaf redness benefits fitness. Manetas described experiments on natural populations of *Cistus creticus*, an evergreen Mediterranean shrub that exists as two morphs, one with green leaves and one in which anthocyanins are induced after bright cool days in winter. The red phenotype displayed lower photoprotective capacity and leaf N concentrations both before and after reddening (Kytridis *et al.*, 2008). Moreover, after induction of anthocyanin accumulation, the photosynthetic capacity of the red morph was considerably reduced compared with the green form, indicating that anthocyanins may not afford adequate photoprotection. A reduced fitness of the red phenotype was indirectly inferred from measurements of leaf fluctuating asymmetry.

Autumnal colours as signals

Simcha Lev-Yadun (University of Haifa, Israel) considered some general features of defence, highlighting its relative nature and the example of colourful thorns and spines as aposematic (warning) signs. The question of signalling is central to the coevolution view of autumn colorations and was a recurrent theme in this meeting. Lev-Yadun also suggested some further possible relationships between red leaves and their predators. Insect camouflage may be undermined by changing foliage colour. If autumn leaves are signalling that they are about to be shed (Lev-Yadun & Gould, 2007) insects may be deterred, as leaf-fall is a significant cause of insect mortality (Faeth *et al.*, 1981). The speaker also speculated on leaf coloration in relation to various kinds of mimicry including Müllerian and Batesian aposematism.

Alan Grafen (University of Oxford, UK) gave the theoretician's view of signalling in biology, and the rationale behind the handicap principle (Grafen, 1990). Signalling occurs when one party (the signaller) conveys some meaningful information lacking to another party (the receiver). Signalling can often be seen as an arms race between the signaller and the receiver. Why, then, a signal should be reliable and the receiver trust the signal has been the central problem of the evolutionary theory of signalling, at least since Zahavi (1975) proposed the 'handicap' principle. Grafen (1990) and others showed that, if a signal is costly to produce (a cost paid in addition to the 'efficacy cost' that is needed simply to display the information unambiguously), then the good signallers would signal at high levels in order to display their higher condition to receivers. Bad signallers, conversely, would accept a lower advantage deriving from the receiver's response to the signal rather than pay the (for them) higher price of displaying a strong signal. More precisely, the requirement for the evolutionary stability of costly signalling is that the ratio of the fitness cost of the signal and the benefit received be lower in individuals giving stronger signal. 'Handicaps' are signals that, although they could be faked in principle, are not convenient to fake. Signals can be honest for other reasons, even at no cost, for example if the signal is an index that cannot be faked (Maynard-Smith & Harper, 2003).

Marco Archetti (University of Oxford, UK) explained how signalling theory applies to the context of autumn colours, pointing out that autumn colours need not be costly. In fact, in the original handicap model of autumn colours (Archetti, 2000), the cost paid at the signalling equilibrium can even be zero. It is also important to remember that the trees signalling at higher levels are not necessarily the vigorous ones. This is true only if vigour (what matters for the tree in the production of the signal) and defences (or nutrition, or what matters for the insects in the choice of a tree) are linked in trees (as in the original model of Archetti, 2000), but this is not necessarily the case (Ougham *et al.*, 2005). If vigour and defences are decoupled other possibilities arise, and the signalling individuals will not necessarily be the vigorous ones. This is still in accordance with the coevolution theory and it is important to understand the consequences of this effect when planning experimental tests.

Simon Leather (Imperial College London, UK) celebrated 'beautiful creatures – sap-sucking insects' and explained the aphid life-cycle. He considered in detail the heteroecious (host-alternating) species that flee from nutritional decline in herbaceous hosts in autumn and take up residence in trees (Archetti & Leather, 2005). Data on the nutritional quality of autumn foliage are limited, although leaf yellowing is a good indicator of the availability of amino acid N released by proteolysis during senescence (Holopainen & Peltonen, 2002). Generally, aphids prefer young tissues (in some cases they may even cause hormonal disturbance in the tissues they feed on, thereby 'engineering youth'), but they favour yellow senescing over mature green foliage. There are examples both of aphids 'engineering' senescence and of plants responding to attack by prematurely senescing. Differential survival rates of the morphs of heteroecious aphids feeding on young, mature or senescent leaves are indicative of a strong adaptive element in the evolution of complex life-cycles (Leather & Dixon, 1981; Kundu & Dixon, 1995).

Thomas Döring (Imperial College London, UK) described trapping experiments designed to test the colour preferences of migrating aphids. A matrix of targets, comprising water-filled Petri dishes painted in a range of blues, greens and yellows, was set up in the field and the numbers of caught aphids counted. The picture in spring was clear – more aphids, of all species, were caught in bright yellow Petri dishes than in green, and blue was largely avoided. When a similar experiment was conducted in autumn, this time including a range of red, orange, pink and brown targets, yellow and green once again caught high numbers of aphids. Relatively low numbers were recorded in brown targets, and lower still in red. The speaker pointed out that combining data on spectral reflectance curves of leaves with colour choice data of aphids obtained in the trapping experiments indicates a low attractivity of red leaves compared with green ones (Chittka & Döring, 2007). The presentation ended, as did many at the meeting, with an acknowledgement that more experimentation is needed.

The subject of Snorre Hagen's (University of Tromsø, Norway) contribution concerned the applicability of the autumnal signalling hypothesis to insect herbivore–tree interactions in subarctic mountain forests of *Betula pubescens*. In this case the herbivores were defoliating moths (Mjaaseth *et al.*, 2005). Mountain birch generally yellows but does not redden in autumn. Early onset of senescence and the development of intense colour in September was negatively correlated with insect damage the following season. There was a positive correlation between physiological stress and reproductive investment on the one hand and insect damage the following season on the other. These studies provide indirect support for the handicap signal hypothesis (Hagen *et al.*, 2003).

Conclusions

In the workshop session, the participants reviewed the evidence for the physiological (photoprotective, antioxidant and plant nutrition) and signalling functions of anthocyanins. It was concluded that there are some things we think we know: aphids are attracted to yellow but not to red, for example. There are some questions we do not have answers to at present: for example, what, in the context of leaf coloration, is the appropriate measure of the fitness of a tree, or an aphid? And there is the uncomfortable fact that there seem to be more hypotheses, and more published reviews and opinion pieces, on the subject of red leaf coloration than sources of good experimental data. To resolve these issues requires tribal barriers to be removed; this stimulating meeting was a first and decisive step in that direction.

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