



Red leaves, insects and coevolution: a red herring?

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W.D. (Bill) Hamilton proposed that coevolution between plants and herbivorous insects explains the bright autumnal colouration of leaves. Accordingly, plants invest in bright signals to reduce their herbivore load, whereas insects use these bright signals to identify less-defended hosts more efficiently. Archetti and Brown have recently revisited this theory by explaining its basic predictions and providing new research perspectives. Their work presents an important basis to our understanding of non-green leaf colouration, provided that alternative adaptive explanations on the photoprotective and antioxidant role of leaf pigments, or their possible function in crypsis to herbivores are incorporated into future research.

The late Bill Hamilton, one of the most important evolutionary theorists of the 20th century, suggested shortly before his death in 2000 a strikingly novel theory to explain the leaf colours of autumn trees as a defence against insect attack [1,2]. This theory was labelled as ‘the coevolution theory’, although here we term it the ‘leaf signal theory’ to distinguish it from the many other hypotheses related to coevolution. A new paper by two of his collaborators further develops these ideas [3] and addresses some of the recent criticisms of the theory [4,5].

Before the introduction of the leaf signal theory, most ecologists regarded leaf colouration as a consequence of leaf senescence preceding abscission and, as such, it attracted little attention. By proposing a new adaptive explanation, Hamilton and co-workers laid out innovative research perspectives [1–3] that triggered experiments [6,7] as well as controversy [4,5]. The key attraction for a wide audience is that the hypothesis touches on several disparate fields: coevolution and signal theory, insect and plant ecology, biochemistry and plant physiology. This is also its main challenge: the predictions of leaf signalling theory have to be tested against other adaptive explanations of leaf pigments before the theory can gain wide acceptance. Therefore, experiments to test the leaf signalling theory must be designed such that other, not necessarily mutually exclusive, hypotheses, such as the photoprotection theory [8–12], suggested first nearly a century ago [13], are taken into account.

Leaf signalling theory posits that bright leaf colouration in autumn serves as a signal to herbivorous insects and reveals the defensive commitment of the individual plant. The fundamental conjecture is that signalling

defensive strength enables well defended individuals to reduce their herbivore load and, at the same time, insects to locate suitable hosts more efficiently. Thus, the basic prediction of the theory is that bright colours are honest signals that enable insects to select less well-defended trees; therefore, trees with bright leaves will suffer a lower level of insect attack than will those that are dull coloured. In this proposed signalling system, less-protected plants are the losers. Such a signalling system requires costs either in the production of the signal or increasing marginal costs to prevent cheating by low-quality individuals. The authors [3] are cautious about how the signal relates to individual quality postulating that the intensity of colouration indicates a plant’s defensive commitment. Such a relationship between secondary compounds acting as feeding deterrents to insects and colouration has been documented in some leaves and fruits [14,15]. Archetti and Brown propose further that honesty-enforcing costs are found (i) in the timing of the signal (i.e. plants that change colour early lose primary production owing to an early cessation of photosynthesis); or (ii) in the intensity of the signal (i.e. it is the colouration itself that costly).

In support of leaf signalling theory, an analysis of the literature documented that species with brighter autumn colouration harboured more species of specialist aphids than did duller-coloured species, suggesting that potentially vulnerable plant species evolved brighter leaf colouration [1]. Moreover, an early onset of autumn colouration in mountain birch *Betula pubescens* correlated negatively with aphid damage in the following season, probably because fewer aphids laid their eggs on individuals that changed colour early in the season [6]. In this species, individuals with bright leaves also had a lower degree of fluctuating asymmetry, supporting the idea that there is a link between plant vigour and the intensity of the signal [7].

Currently, this theory is limited to leaf senescence in spite of the fact that coevolution between insects and plants has also been suggested as an explanation of the occurrence of anthocyanins in young unfolding leaves [16]. Here, red leaves were assumed to be cryptic to insects rather than conspicuous, as postulated by leaf signalling theory. That both hypotheses assume different functions of red leaf colouration is attributable to the variation in the occurrence of a red-light sensitive receptor type in insects. Although red-light sensitive receptors have evolved independently in some species of four insect orders (Odonata, Hymenoptera, Lepidoptera and Coleoptera) [17], most insects are not particularly sensitive to red light, and

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Glossary

Anthocyanins: water-soluble red, rarely blue, pigments found in the cell vacuoles of both juvenile and senescing leaves of many plant species.

Antioxidant: prevents or slows the breakdown of a substance by oxygen, especially reactive oxygen species.

Carotenoids: highly unsaturated lipid-soluble yellow–red pigments produced by plants and fungi.

Photoinhibition: a light-induced stress reaction embracing all reversible and irreversible phenomena that lower the efficiency of photosynthesis.

Reactive oxygen species (ROS): includes all molecules containing oxygen with an unpaired electron (i.e. a free radical); generally reactive with other substances and often induce damage to tissue.

hence leaf crypsis was proposed. By contrast, leaf signalling theory has focussed on aphids, in which the red-light sensitive receptors have not yet been found. There is a clear need for more comparative data on the colour vision of herbivorous insects before refuting either of the two hypotheses.

Plant physiology

In their paper, Archetti and Brown [3] deal only with insect and plant ecology and assume that plant physiology might not explain the observed variation in leaf colouration. It is unfortunate that alternative adaptive hypotheses [8–12] are neglected, because plant pigments can signal to animals and simultaneously serve physiological functions within the leaf. How then can we disentangle these different functions? Here, we summarize how photoprotection might affect leaf colouration.

Tissue involved in photosynthesis is susceptible to damage by high illumination because it is unlikely to release as heat excess energy that cannot be used for photosynthesis. Excess light quanta might instead result in the formation of REACTIVE OXYGEN SPECIES (ROS) (see Glossary). To avoid damage to tissues by these and other substances, many plants rely on a strategy called PHOTONHIBITION [18], which can be induced by excess light, ROS and low temperatures [19]. Because photoinhibition might lower productivity and growth, it is crucial for plants to reduce the frequency of photoinhibiting reactions, especially those that are irreversible. This is achieved by the photoprotective and ANTIOXIDANT role of the CAROTENOIDS and ANTHOCYANINS pigments, which are responsible for the yellow–red hues in leaves [5]. These pigments intercept excess light quanta that are otherwise absorbed by chlorophyll b, and therefore provide a ‘sunscreen’ [8]. Moreover, both pigments also act as scavengers of ROS, preventing damage to the tissue involved in photosynthesis [9,20]. Several recent studies highlighting these functions challenge the basic assumptions of leaf signalling theory.

Signal timing

The timing of the signal is not necessarily an indicator of honesty-enforcing costs: an early shedding of leaves might be adaptive in cold climates because a plant often consumes more CO₂ than it produces owing to frequent temperature-induced photoinhibition [19]. Moreover, and contrary to the assumptions of Archetti and Brown [3], photosynthesis does not necessarily cease with the onset of colouration. On the contrary, the photoprotective role of anthocyanins might lower the frequency of cold-induced

photoinhibition, resulting in constantly high levels of photosynthesis in red leaves at low temperatures [10]. Anthocyanin accumulation might also result in increased, rather than decreased, levels of photosynthesis in red compared with green leaves at variable light conditions [8]. Thus, variable illumination combined with low temperatures in autumn alone might explain the synthesis of anthocyanins in autumn.

Signal intensity

The intensity of the signal is not a reliable indicator of honesty-enforcing costs: leaf senescence is a tightly controlled process for transferring nutrients, particularly nitrogen and phosphorus, from leaves to perennial tissue. Species with a brighter autumnal colouration appear to retain more nutrients from the leaves than do duller coloured species [21,22], potentially explaining the inter-specific variation in leaf colouration observed by Hamilton and Brown [1]. Only the photosynthetic tissue that is well protected against frequent photoinhibition can supply sufficient energy for nutrient translocation. This translocation accounts for most of the nitrogen and phosphorus reservoir that influences growth and reproduction in the following year [11]. Thus, the positive correlation between the intensity of autumnal leaf coloration and plant vigour in mountain birch [7] might be attributable to the extent of nutrient recovery rather than to signalling defensive commitment alone. Consequently, the costs of signal production (i.e. forming anthocyanins and carotenoids) have to be balanced against the benefits of nutrient recovery and photoprotection.

In addition, anthocyanins act as antioxidants scavenging free radicals in leaves [9]. In autumnal leaves, this antioxidant role is particularly important because the process of nutrient recovery requires the breakdown of leaf material, causing an increased risk of forming ROS. The oxidative stress increases further owing to low temperatures combined with a high light level, which might ultimately lead to the destruction of the photosynthetic apparatus [23]. Interestingly, and owing to this antioxidant role, leaf colouration might be correlated with insect herbivory, not as a signal to divert insects, but as a result of mechanical injury by herbivorous insects preventing further oxidative damage from the uncontrolled breakdown of substances in already injured tissue [9]. By shaping the elemental processes of photosynthesis, nutrient recovery and protection of tissue against oxidative stress, the physiological role of anthocyanins and carotenoids contributes fundamentally to plant fitness. Consequently, these adaptive explanations, rather than leaf signalling, might explain the variation in leaf colouration.

Conclusion

Thanks to Hamilton and co-workers, the inter- and intraspecific variation in autumnal leaf colouration has been brought to the attention of both ecologists and evolutionists. As yet, it is unclear which of the alternative hypotheses will explain the phenomenon. To understand the phenomenon from an evolutionary perspective, we must embrace all contexts in which non-green leaf colouration occurs. Red pigmentation is found not only

Box 1. Research perspectives that integrate physiology and signalling

The main challenge to the study of leaf colouration is to control for the different functions of pigments, summarized as: (i) signalling to insects; (ii) acting as antioxidants; and (iii) protecting leaf organelles via the interception of light quanta during adverse environmental conditions and nutrient translocation. (ii) and (iii) can be merged into the photoprotection theory. Here, we outline a few avenues for separating the leaf signalling and photoprotection theories.

- A promising model system is an intraspecific comparison between anthocyanin-deficient mutants and wild-type individuals [10,11]. An easy test of leaf signalling theory is to ask whether the insect load is greater in mutants than in wild-type individuals. Likewise, determining whether the rate of photosynthesis and nutrient recovery are generally higher under adverse conditions in wild-type individuals tests the photoprotection theory. The comparison between wild types and mutants also serves as a model to assess the costs of foliar pigmentation. In wild types, the metabolic costs during anthocyanin accumulation can be balanced against the relative benefits of nutrient recovery (compared with mutants).

- Another useful approach is to manipulate environmental conditions during senescence and study their influence on the intensity of leaf colouration. Photoprotection theory expects a strong influence, especially by shading and temperature, whereas, according to Archetti [2], the putative signalling system between plants and insects collapses if the environmental effects are too strong. For all experiments, leaf colouration should be recorded with a spectrometer to enable high repeatability and detailed measurements to be taken. So far, most researchers have scored leaf colouration according to categories based on the human eye. Although these indices might be easy to use, they remain subjective, impeding easy replication of published results. Using a spectrometer also enables fine-tuned correlation of the intraspecific variation in the intensity of foliar pigmentation with the efficiency of nutrient recovery.

- Quantifying the foliar concentrations of insect-deterrent secondary compounds, such as tannins [15], in brightly coloured leaves is essential when testing the central assumptions of leaf signalling that defensive commitment is indicated by leaf colouration. If spectrometric readings are taken, the concentrations of secondary compounds can be compared with the intensity of the signal.

- Experiments of leaf signalling theory (bright leaves during senescence) should be extended to encompass all instances of bright leaf colouration (young leaves and understorey plants) to facilitate our understanding of the evolutionary origin of the phenomenon. The fact that anthocyanins are present in plants growing under the variable light conditions of the forest floor, where sun flecks briefly interrupt otherwise dim light conditions, as well as in both transitory stages of leaves (construction and destruction of organelles) might indicate a common photoprotective role in all occasions.

during senescence, but also in unfolding new leaves and in shade-adapted understorey plants. Whether its function is the same in all these instances should be the focus of future work (Box 1). All future research must determine which of several possible ecological and physiological functions will explain the occurrence of non-green leaves. In doing so, we should not rely on our tendency to want to believe in simple and attractive explanations to complex phenomena.

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