

Autumn leaves seen through herbivore eyes

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Why leaves of some trees turn red in autumn has puzzled biologists for decades, as just before leaf fall the pigments causing red coloration are newly synthesized. One idea to explain this apparently untimely investment is that red colour signals the tree's quality to herbivorous insects, particularly aphids. However, it is unclear whether red leaves are indeed less attractive to aphids than green leaves. Because aphids lack a red photoreceptor, it was conjectured that red leaves could even be indiscernible from green ones for these insects. Here we show, however, that the colour of autumnal tree leaves that appear red to humans are on average much less attractive to aphids than green leaves, whereas yellow leaves are much more attractive. We conclude that, while active avoidance of red leaves by aphids is unlikely, red coloration in autumn could still be a signal of the tree's quality, or alternatively serve to mask the over-attractive yellow that is unveiled when the green chlorophyll is recovered from senescing leaves. Our study shows that in sensory ecology, receiver physiology alone is not sufficient to reveal the whole picture. Instead, the combined analysis of behaviour and a large set of natural stimuli unexpectedly shows that animals lacking a red photoreceptor may be able to differentiate between red and green leaves.

Keywords: anthocyanin; aphid; coevolution; colour-opponent mechanism; crypsis; herbivory

1. INTRODUCTION

The autumn colours of many trees in the temperate regions are one of nature's most spectacular phenomena. Yet until recently, surprisingly little research has been dedicated to understanding the adaptive significance of colour change in autumn. Partly, colour changes in autumnal foliage occur simply as a consequence of chlorophyll breakdown, which unmasks other pigments such as the yellow carotenoids. These may therefore not serve any particular autumnal function. However, anthocyanins that are responsible for red leaf coloration are newly synthesized in autumn (Sanger 1971), just before leaves fall to the ground. Many researchers have sought the adaptive value of anthocyanins in plant physiological functions, e.g. in photoprotection, but currently even plant physiologists do not unanimously agree on this (Manetas 2006; Ougham *et al.* 2008).

Another, more recent idea is that red could be a signal of the tree's status to insects that migrate to the trees in autumn. According to this coevolution theory (Archetti 2000; Hamilton & Brown 2001), trees with red leaves have better defences against herbivores, or poorer nutritional quality or other characteristics that induce a lower fitness in the insects; herbivorous insects migrating to the trees in autumn are expected to avoid red leaves and preferentially colonize trees with green leaves. In this scenario, tree colour and colour preference by herbivores, therefore, may have coevolved in an arms race: autumn colours as an adaptation of the trees to reduce their parasite load, and preference for green in insects as an adaptation to find the most suitable host trees. Looking for receivers of the trees' potential

signal, the authors of the coevolution hypothesis turned to aphids, because many host-alternating aphid species migrate in large numbers from their summer hosts back to primary host trees during the period that broadly coincides with autumnal leaf colour change. The main cost that aphids inflict on trees then occurs only in the following spring when the new generation of aphids hatches from the overwintering eggs that were laid in autumn.

Over the last few years, the coevolution hypothesis has prompted an extremely prolific and heated debate on the evolution of autumnal leaf coloration (Lee 2002; Wilkinson *et al.* 2002; Manetas 2006; Chittka & Döring 2007; Ougham *et al.* 2008). However, many of the ideas that have since been published in support or objection of the idea have mainly rested on theoretical considerations or on rather isolated observations, whereas the little experimental evidence gathered so far has remained inconclusive. Surprisingly, one of the key questions to decide whether the coevolution hypothesis could be correct has remained untackled: would aphids that are about to choose their host respond to red leaf colour in any different manner from that they would to green leaves? Further motivation for answering this question came from an agricultural perspective, as a deeper insight into the response of aphids to colours could help to develop aphid control strategies that make use of distinct crop leaf colours (Müller 1964; Prokopy & Owens 1983; Prokopy *et al.* 1983).

To approach the question of leaf colour attractiveness for aphids, we set up a large colour-choice experiment in the field, using water traps painted with 70 different colours in autumn 2007, systematically varying the colours in the range of blue, green, yellow, orange and red. Using the aphid catch, spectral reflectance measurements of the colour traps and information on the sensory physiology of aphids (Kirchner *et al.* 2005; Döring & Kirchner 2007),

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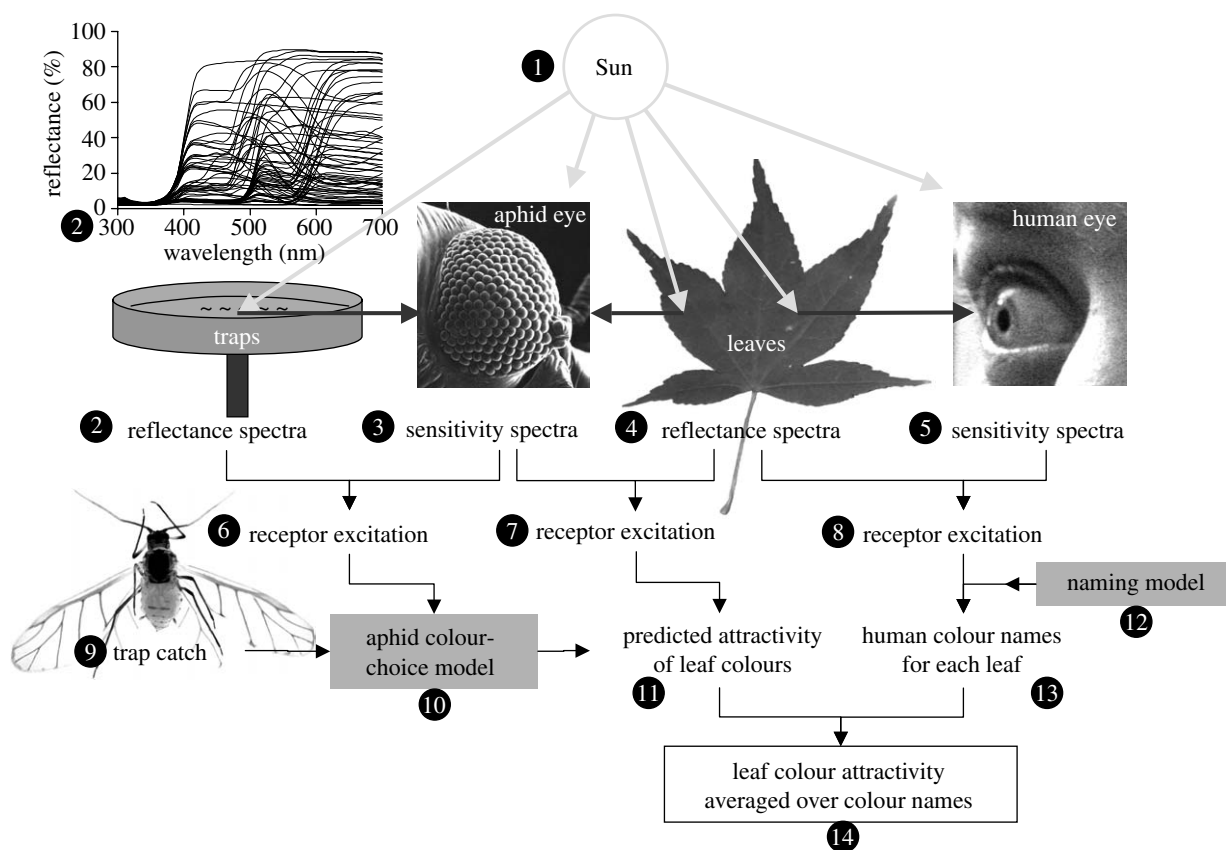


Figure 1. Overview of experimental approach. For an explanation of numbers, see §2.

we were then able to build a simple mechanistic colour-choice model for these insects, which allows assessing the attractivity of any colour for landing aphids. By applying this model to a large number of tree leaf spectra of different colours, we could then assess the relative attractivity of red versus green tree leaves for aphids.

2. MATERIAL AND METHODS

(a) General approach

To assess the attractivity of leaf colours for herbivores, we needed to address three problems. First, leaf colour is likely to be correlated with other properties of the leaves, e.g. red leaves may emit a different profile of plant volatiles than green ones, and may smell differently to the herbivores. Therefore, the direct use of coloured leaves in choice tests risks confounding the property ‘colour’ with other factors. Second, ‘red’ and ‘green’ are not necessarily biologically relevant categories at all, because humans and insects differ strongly in their colour vision abilities and the insects’ responses are unlikely to correspond to colour categories that are solely based on human colour perception (Chittka & Döring 2007); therefore colours needed to be assessed in an objective way, i.e. as reflectance spectra measured by a spectrophotometer. Third, however, in the entire literature concerning the coevolution hypothesis on autumn leaf coloration, human colour categories were used to describe trees and leaves; therefore, to be able to compare our results with previous findings, we needed a reproducible and objective way of linking reflectance spectra to human colour classification of leaf colours. This was also necessary because a leaf that one observer calls ‘red’ might be called ‘orange’ or ‘brown’ by another observer (Roberson *et al.* 2005).

To deal with the first two problems, we aimed to generalize the response of aphids to colours and develop a colour-choice model that would allow the application to leaf colours as well, and would thereby make it possible to assess the attractivity of red versus green leaves without the possible confounding effects of other leaf properties. For developing the model, we set up an array of painted water traps and then used the illumination spectrum of the Sun (figure 1, black-encircled number (1)), the reflectance spectra of the traps (2) and the sensitivity spectra of aphid photoreceptors (3), to calculate the photoreceptor excitations (6) that each trap would generate in an aphid eye. Using these receptor excitations as a set of explanatory variables and the normalized number of aphids in each trap (9) as the response variable, it was possible to build a general aphid colour-choice model (10). This model was then applied (11) to tree leaf colours by determining the corresponding receptor excitations (7) that the spectra of the leaves (4) would generate in the aphid photoreceptors.

For addressing the third problem, we aimed to have a reproducible measure of the colour classification of the tree leaves into colour categories such as red and green. Using the illumination spectrum of the Sun (1) and the reflectance spectra of the leaves (4) again, but this time in conjunction with the spectral sensitivities of human photoreceptors (5), we could calculate the receptor excitations in humans (8) for each leaf. By feeding these excitation values into a recently developed objective colour-naming model (12), we could translate each leaf spectrum into an objective colour name (13). The attractivity values of leaves (11) that had the same name (e.g. red) could then finally be averaged (14), so that the results are independent from how a particular human observer would assess or name the colour of a leaf. We should emphasize that human colour names such as red and green

remain biologically completely irrelevant in the aphid-tree system; the purpose of the classification is just to enable us to link our results with the predictions and findings of previous studies that were solely based on colour names. A more biologically relevant measure of colour would be the pigment content of the leaves.

(b) Trapping and insect identification

The trapping experiment was set out on Silwood Park campus (near Ascot, Berkshire, UK), on a bare sandy soil. For trapping the aphids, we first built a trap stand for each trap by glueing a Petri dish cover (diameter 14.25 mm and rim 9 mm high) onto a 40 cm long black-painted wooden rod (2 cm diameter). The rods were then stuck into the soil so that the Petri dish cover, now functioning as holder for a Petri dish of 14 cm diameter, was approximately 25 cm above the ground. Painted Petri dishes (rim height 19 mm) were then put onto the trays, and filled with tap water and 2 ml of approximately 1 : 20 diluted odourless and colourless detergent (Lipsol from Bibby Sterilin Ltd, UK) each. We produced 70 different paints by mixing varying amounts of water-based masonry base paints (blue, green, yellow, red and black: 'Weathershield smooth masonry extra deep' from ICI Dulux; white: water-based 'Marbletex Smooth Brilliant White' from Macpherson, UK). Each of the 70 paints was applied to two Petri dishes with two coats.

The dates of catching were 11–15 October 2007 and 18–22 October 2007. In each of the two periods, a different randomization layout for the 140 traps was used to reduce spatial bias. The spatial layout was a 2 × 2 m grid with four rows of 35 traps. Both long sides of the experimental area were neighboured by strips of bare soil at least 8 m wide. The field site was freshly rotavated before the trapping period to produce a constantly homogeneous vegetation-free background for the whole trapping period. All aphids were identified to species level where possible.

(c) Spectral reflectance measurements of leaves, traps and soil

Autumnal leaves were taken from 113 tree species in total (47 genera) at four sites in the UK (Kew Gardens, London; Harcourt Arboretum, Oxford; Silwood Park, Berkshire; and the national fruit tree collection, Brogdale Horticultural Trust, Faversham), as well as two sites in Germany (near Witzenhausen, and at the Forestry Department Arboretum, University of Göttingen). Only leaves that were still attached to trees were selected. Leaf spectra were always measured within a few hours of collection. To prevent desiccation, leaves were placed into envelopes directly after picking until spectral measurement. Spectral measurements were performed on the upper surface of 977 leaves. For most leaves, two or more spectra were taken, resulting in a total of 3231 leaf spectra.

Reflectance spectra were measured either with a RAMSES-ARC spectrophotometer (from TriOS GmbH, Oldenburg, Germany), or an AvaSpec 2048 spectrophotometer (from Avantes, The Netherlands), against a BaSO₄ white standard. The RAMSES spectrometer (spectral range 320–950 nm, 5 nm steps) was used for integration over larger areas (approx. 10⁻⁴ to 10⁻¹ m²), and for wet or rough objects (i.e. soil and water-filled traps). The Avantes spectrometer (range 290–800 nm, 0.29 nm steps) with a smaller integration area (approx. 10⁻⁶ to 10⁻⁵ m²) was used for most leaves. Spectra were taken from traps filled with tap water and Lipsol solution.

(d) Calculations of insect photoreceptor inputs and human colour names

For building a model of colour response in aphids, which is based on the insects' colour perception, it is first necessary to translate trap spectra into photoreceptor excitations, i.e. to calculate the photon catch that a trap would produce in an aphid's photoreceptor. Generally, the photon catch P that a stimulus s (i.e. a trap or leaf) elicits in a photoreceptor R is calculated as

$$P_R(s) = \int I_s(\lambda) S_R(\lambda) D(\lambda) d\lambda / \int I_b(\lambda) S_R(\lambda) D(\lambda) d\lambda,$$

where $I_s(\lambda)$ is the reflectance spectrum of the stimulus s ; $S_R(\lambda)$ is the sensitivity function of the photoreceptor R ; $D(\lambda)$ is the illumination spectrum; and $I_b(\lambda)$ is the reflectance spectrum of the background b against which the stimulus s appears (Chittka 1996).

The standard sunlight spectrum D65 was chosen for $D(\lambda)$. The background spectrum, i.e. the spectrum of the bare soil at the trap site, was determined as the average of three spectral measurements on 13 October 2007 taken with the RAMSES spectrophotometer at 50 cm distance between soil and photometer against the BaSO₄ white standard.

For two aphid species, electrophysiological experiments have indicated the position of the peak spectral sensitivity (close to 530 nm; Kirchner *et al.* 2005; Döring & Kirchner 2007). These measurements in conjunction with sensitivity templates (Stavenga *et al.* 1993) allow the modelling of photoreceptor sensitivity curves $S_R(\lambda)$. We used these templates to calculate sensitivity curves with peaks from 320 to 610 nm in 10 nm steps. With a fixed ratio between half-max bandwidth $\Delta\lambda$ and peak wavelength λ_{\max} ($\Delta\lambda/\lambda_{\max} = 0.18$), the breadth of the sensitivity curve was smaller at shorter than that at longer peak wavelengths, reflecting observed data (Peitsch *et al.* 1992). All data presented refer to receptors without a secondary peak in the UV, but no inconsistencies were found with alternative calculations that included such a beta peak at 350 nm. In our model, G and B represent the photon catches of the green ($P_G(s)$) and blue ($P_B(s)$) photoreceptors, respectively.

For the human visual system, each leaf was placed in a human colour-name category via the calculation of CIELAB values (L , a , b) as follows (Sharma & Trussell 1997). First, the photon catches P [X , Y , Z] produced by each leaf f in each of the three human photoreceptors H [blue, green, red] were calculated with $P_H(f) = \int I_f(\lambda) S_H(\lambda) D(\lambda) d\lambda$. The sensitivity functions $S_H(\lambda)$ were taken from the CIE 1931 standard human observer data. Then, L , a and b were calculated as $L = 116Y^{1/3} - 16$, $a = 500((X/0.9504)^{1/3} - Y^{1/3})$ and $b = 200((Y^{1/3} - (Z/1.089)^{1/3})$.

With the additional measures $C^* = (a^2 + b^2)^{0.5}$ ('chroma') and $h = \arctan(b/a)$ ('hue'), the leaves and traps could be categorized into human colour names following an empirical colour-naming model (Lin *et al.* 2001). Owing to slight overlapping of the colour-name boundaries in this model, a minority of leaf spectra ($N=822$) was placed into more than one category by the model (e.g. 'yellow' and 'green'); these were excluded from all subsequent analyses, so that only leaf spectra with unambiguous leaf colours ($N=2409$) remained in the dataset.

(e) Statistical calculations and model development

All statistical calculations were performed with the program R v. 2.6.1 (R Development Core Team 2007). To build the colour-choice model, we first calculated the response variable

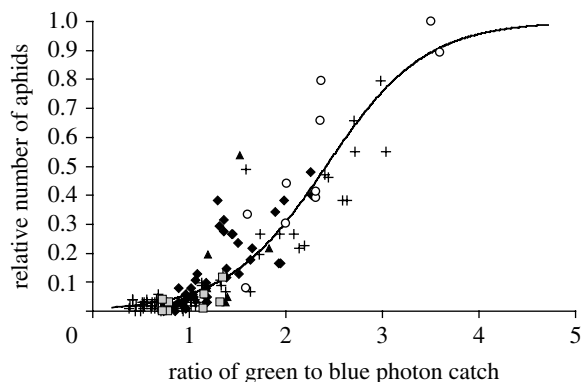


Figure 2. Aphid catch (y) in coloured traps, relative to the maximum catch ($N=102$), and plotted against $x=G/B$, with G and B being the photon catches that a trap will elicit in green and blue photoreceptors, respectively. Symbols indicate colour names of traps (diamonds, green; triangles, brown; squares, red; circles, yellow), while all other traps (e.g. blue) are represented by crosses.

y as the number of aphids n_t in a trap t relative to the maximum number of aphids n_{\max} that had been caught with one trap ($y=n_t/n_{\max}$). We used a generalized linear model with quasi-binomial errors, after overdispersion was observed with binomial errors (Crawley 2007).

For the calculation of G , we chose the peak position of the green receptor at $\lambda_{\max}=530$ nm according to the available electrophysiological data (Kirchner *et al.* 2005; Döring & Kirchner 2007). The position of λ_{\max} of the short wavelength photoreceptor was then found by testing each of the modelled photoreceptors in the range of 320–490 nm (i.e. UV and blue) for minimal residual deviance in the colour-choice model. The least deviance was found with a short wavelength photoreceptor peaking at $\lambda_{\max}=460$ nm.

In the analysis of leaf attractivity, we considered leaves per plant species and spectral measurements per leaf as pseudo-replications. Therefore, we used a nested model with measurements within leaves within plant species as random effects.

3. RESULTS

In the trap experiment, a total of 2109 winged aphid individuals from 56 aphid taxa were found. The catch was dominated by *Rhopalosiphum padi* L. (24%), *Sitobion fragariae* Walk. (22%), *Aphis fabae* Scop. (9%) and *Myzus persicae* Sulz. (8%), all of which are host-alternating species.

Evidence from laboratory experiments indicates that colour preference in aphids probably follows a colour-opponent mechanism (Döring & Chittka 2007), i.e. landing behaviour in aphids is coupled to a neural mechanism with antagonistic input from the green versus a short wavelength (blue or UV) photoreceptor. Therefore, we looked at various mathematical representations of colour-opponent mechanisms as explanatory variables and tested several alternative models, including piecewise linear regressions, linear difference functions or Gompertz functions of photoreceptor inputs. The chosen model was the most economic (i.e. least number of parameters), which was able to predict saturation effects (i.e. to incorporate a maximum relative attractivity). This model uses the expression G/B , i.e. the ratio between the photon catch G of the green photoreceptor and the photon catch B of the blue photoreceptor, as a representation of the colour-opponent mechanism.

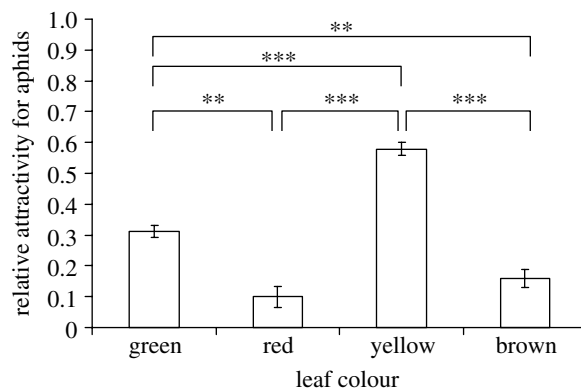


Figure 3. Attractivity of differently coloured tree leaves for aphids as assessed by the colour-choice model from figure 2 and grouped according to an empirical colour-naming model; statistical significance of pairwise (local) comparisons indicated by asterisks over brackets (** $p<0.01$; *** $p<0.001$); red and brown are not significantly different.

Thus, the normalized aphid catch in the coloured traps followed a logistic model (figure 2), when plotted against $x=G/B$, where G is the photon catch elicited by a trap in a green photoreceptor (maximal sensitivity at $\lambda_{\max}=530$ nm), and B the photon catch of the same trap for a blue photoreceptor ($\lambda_{\max}=460$ nm). When all aphid species were pooled, the model had the shape $\ln[y/(1-y)]=ax+b$, with $a=1.95\pm 0.10$ and $b=-4.71\pm 0.18$ (mean \pm s.e., $r^2=0.81$, $n=140$, $p<0.001$). When the analysis of colour-choice behaviour was performed with single aphid species, similar logistic models could be used to model the catch of each of the four dominant species. According to an ANCOVA, the parameters a and b did not differ significantly between *R. padi*, *A. fabae*, *M. persicae* and the overall catch, but *S. fragariae* was significantly different from all other aphid species ($a=0.91\pm 0.21$, $b=-3.91\pm 0.40$). Nevertheless, we pooled the data from all aphid species for the presentation of the colour-choice model, because all separate species models followed the same general logistic shape (also see below).

When the trap colours were subjected to the colour-naming model, points representing red traps formed a cluster with low values of both G/B and normalized aphid numbers, whereas green traps showed a larger variability on both axes (figure 2). The maximum catch was found in a yellow trap.

For the colour-choice model, we further tested whether residuals e_i between predicted values \hat{y} and observed values y_i of the aphid catch ($e_i=y_i-\hat{y}$) could be explained by further colour information in the spectra of the colour traps. This was done by calculating Pearson's correlation coefficient r between the residuals e_i and the photon catch P_R for each trap and for each of the modelled photoreceptors R (peak sensitivities at $\lambda_{\max}=R$). The maximal correlation was $r=0.011$ at 530 nm ($p=0.89$, not significant) and the minimal correlation was $r=-0.058$ at 410 nm ($p=0.50$, not significant). There was also no additional correlation between the residuals and trap brightness. Therefore, we conclude that the model is complete in terms of spectral input information.

When the reflectance spectra of the tree leaves were fed into the colour-choice model for aphids, and the resulting attractivity values were averaged across the colour names, green leaves showed a significantly higher attractivity than

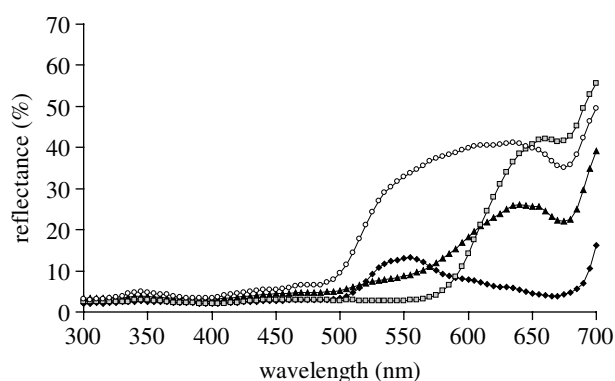


Figure 4. Averaged reflectance spectra of 360 brown, 1103 green, 160 red and 786 yellow leaves; symbols indicate colour names and are the same as given in figure legend 2.

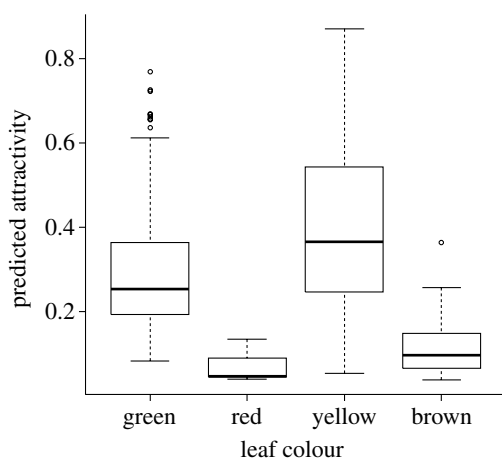


Figure 5. Attractivity of differently coloured bird cherry leaves for aphids. Colours as named by humans according to a colour-naming model. Boxes and whiskers represent the quartiles and the range of the data, but exclude outliers which are depicted by open circles.

red leaves, but the colour of yellow leaves was even more attractive than that of the green leaves (figure 3). The corresponding averaged leaf spectra of the four different colour categories are displayed in figure 4. Because *S. fragariae* showed significantly different parameters from the other species, we tested whether the model derived from *S. fragariae* would generate qualitatively different attractivity values. However, this was not the case: the correlation between attractivity values derived from the all-species model and the *S. fragariae* model was very high ($r^2=0.91$), and the order of leaf colour attractivities (red < brown < green < yellow) remained the same, indicating that our results are relatively robust against changes in the model parameters.

Although leaf colours that appear red, green, brown and yellow to a human observer were significantly different in their attractivity for aphids (figure 3), the variability of spectral reflectance within each of the human colour names still resulted in some overlapping of attractivity ranges. As an example, we show the attractivity of differently coloured leaves in one of the tree species, the bird cherry (*Prunus padus*; figure 5).

4. DISCUSSION

In the colour trap experiment, we were able to build a reliable colour preference model for winged aphids in

autumn. While all of the four dominant species we found are host alternating, it is possible that not all individuals were morphs that do indeed migrate to their winter hosts. However, this is unlikely to influence the aphids' general colour-choice pattern, or, particularly, the application of the model to the leaf spectra.

We showed that the model, based on a colour-opponent mechanism of a green photoreceptor against a blue one, is complete in terms of spectral information in the traps. (This includes the UV reflectance, which is negligibly low both in the traps and in the leaves.) For this reason, the only colour information relevant for the aphids is that on the *G/B*-axis (figure 2).

This means that some green traps (with small *G/B*-values) would be confused with red traps, i.e. they would appear to have the same colour. At the first glance, this is not surprising, because all aphid species tested so far in physiological experiments have been shown to lack a red receptor (Kirchner et al. 2005; Döring & Kirchner 2007). Strictly speaking, animals lacking a red receptor, i.e. the apparent majority of insect species (Briscoe & Chittka 2001), cannot discriminate between red and green stimuli by their colour, because in a test with monochromatic lights they would not be able to distinguish between relatively bright red and relatively dark green lights, thereby confounding intensity and wavelength. However, insects rarely, if ever, encounter monochromatic lights in nature.

Instead, with the realized broad-range reflectance spectra of leaves, the outcome of our analysis is surprising and in contrast to expectations (Döring & Chittka 2007): on average, red leaves are indeed distinguishable from green by colour, because they show lower *G/B*-values than green leaves. That is, on the whole, red and green leaves (and traps) are significantly different on the colour axis that is relevant for aphids. As a consequence, we found that overall fewer aphids landed in red than in green traps and, similarly, 'average leaf red' is less attractive to aphids than 'average leaf green'.

However, the colour-choice model also shows that aphids do not actively avoid red leaves. In a scenario involving avoidance, we would expect that the landing response to pure red would be smaller than that to red mixed with black, but we did not find such an effect; that is, for this subset of red traps the correlation between red reflectance (at 640 nm) and aphid numbers was not negative (Pearson's $r=0.37$, d.f. = 10, $p=0.24$). Furthermore, for flying away from a colour, the landing response would need to be linked to an inhibitory sensory input, in our case the blue component (*B*) in the colour-choice model; but in red leaves and traps, variation in spectral reflectance does not occur in the blue spectral domain. Instead of active avoidance of red leaves by aphids, we find that red leaves are just less attractive than green ones.

However, our results do thereby still lend support to the prediction of the coevolution theory of autumn leaf colours, that aphids which colonize trees with red autumn colours would show a preference for trees with green over those with red foliage. This is of course only a necessary but not a sufficient condition for the coevolution hypothesis, and it is therefore essential to ask whether alternative hypotheses make similar predictions, i.e. a preference for green over red. Note that we restrict this question to the colour red: it had been suggested that yellow could also be an autumnal

warning signal to insects (Hamilton & Brown 2001), but as we have shown this is clearly not the case for aphids, for which yellow is indeed more attractive than green (see also Chittka & Döring 2007).

The remaining crucial question is therefore whether or not red leaf colour has evolved as a signal to herbivores. The first condition for red to be a signal is that it would lead to an advantage for the tree as a result of the behaviour of the insects. As we have shown, this prediction is consistent with our data: the colour red is likely to lead to fewer aphids landing, thereby possibly resulting in an advantage for the red tree (see below). The second condition for the colour being a signal is that the behaviour of the receiver must have evolved as a consequence of the signal. This second part is more difficult to test, and our data do not allow us to directly decide this question. Importantly, the lack of a red photoreceptor alone is not an evidence that the behaviour of aphids did *not* evolve in response to leaf colours, because on average aphids would still be able to distinguish green from red based on the *G/B*-ratio.

Given the high attractivity of yellow, an alternative idea emerging from our results is that red leaf coloration could serve to conceal the attractive yellow colour that is unmasked when chlorophyll is recovered from senescing leaves. Instead of advertising a high defence status as in the coevolution hypothesis, trees would hide away their resources from the insect herbivores. In this scenario, red would not be a signal: although red trees would benefit from the lower attractivity for herbivores, the response of aphids would not have evolved as a consequence of the colour variation among trees. A way to decide whether colour preference in aphids evolved as a consequence of leaf colour is to investigate aphid fitness on trees with autumn-green and autumn-red leaves. If aphids perform equally well on both, or even better on the autumn-red trees, red leaf colour would unlikely to be a signal, but could instead be cryptic, by masking otherwise yellow leaves. Another way of approaching this question is to look at colour preference across different herbivorous insect species: if behavioural preference of green over red is observed in many non-autumn-migrating herbivore species, this might be a hint that this preference has not evolved specifically in response to red autumn colour in trees.

In any case, however, both the leaf crypsis idea and the originally hypothesized coevolutionary link depend on the attractivity of 'real' leaves to aphids: if leaf colours are indeed correlated with aphid-detectable odour profiles or other leaf properties, the next necessary step on the way of scrutinizing hypotheses on the evolution of autumn colours is a choice experiment that can disentangle visual from other cues. Furthermore, the plausibility of these ideas depend on how strongly the landing rates of aphids in autumn are indeed translated first into the number of eggs laid on the tree, and further into the relevant damage to the tree in spring (Döring & Hardie 2007). However, for some aphid species that are considered pests on cultivated tree species, there is at least indirect evidence that aphid egg numbers are indeed related to tree damage in spring (Gimingham *et al.* 1926; Kehrli & Wyss 2001; Blommers *et al.* 2004; Graf *et al.* 2006; Barbagallo *et al.* 2007). Moreover, autumn flight activity of host-alternating aphids seems to be reasonably well correlated with the number of aphids that colonize their secondary

host in the next spring after emigrating from their primary tree hosts (Way *et al.* 1981).

While this issue needs to be tested more thoroughly, damage is certainly inflicted (more immediately) by aphids landing on annual crops (Van Emden & Harrington 2007). Therefore, our findings may also be relevant for aphid control, as the attractivity of plants for herbivores during host searching is a key factor determining later population levels and plant virus transmission rates. Based on our results, the informed selection of leaf colours, using existing colour variation within crop species, could therefore serve as a tool for controlling aphids.

Whatever the effect of aphids on trees or annual crops, the opposite perspective of this plant-herbivore relationship is equally interesting. The consistently low attractivity of red leaves raises the question why aphids have not developed a red receptor that overcomes the low attractivity of red leaves and increases the probability to land on red-leaved hosts in autumn. One possible explanation is that red trees might indeed be less advantageous for aphid fitness, as the coevolution theory predicts (see above). Alternatively, there could be relatively strong constraints for changing spectral sensitivity, let alone for developing an additional red receptor (Briscoe & Chittka 2001), although recent evidence has suggested that, in principle, additional red vision is perhaps less difficult to develop than previously thought (Skorupski & Chittka 2008).

The possibility of differences between aphid species in their colour-choice behaviour suggests that our findings may be limited to those aphid species we found in the traps; therefore, more data on leaf colour choice of aphids in autumn is certainly needed. However, as we have shown, our conclusions regarding the comparatively low attractivity of red leaves are robust against such variations, unless an additional red receptor is used by the herbivores.

Our results emphasize that in sensory ecology, the physiology of the receiver alone, in this case the absence of a red receptor, is not sufficient to reveal the whole picture. Instead, the consideration of a large number of natural stimuli has unveiled an unexpected finding, the colour differentiation between red and green leaves by herbivorous insects that lack a red photoreceptor.

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