

# Insect herbivory may cause changes in the visual properties of leaves and affect the camouflage of herbivores to avian predators

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## Abstract

'Cry for help' hypothesis predicts that attraction of predators with chemical or visual cues can decrease insect damage of plants. Visual cues involve changes in photosynthetic activity and the reflectance of leaves, and there is some evidence that birds may use these changes as foraging cues. However, changes in the visual properties of leaves have not been quantified and it is not known how birds see these changes. We also presented and tested a new 'reduction in camouflage' hypothesis (not mutually exclusive with 'cry for help') stating that herbivore-mediated changes in leaf colour can increase the conspicuousness of herbivore against leaves. To define changes in the visual properties of leaves, their detectability to birds, and whether these changes affect the conspicuousness of herbivore, we manipulated the level of herbivory in silver birch trees (*Betula pendula*) with autumnal moth (*Epirrita autumnata*) larvae, and used blue tit (*Cyanistes caeruleus*) vision models to images of leaves and larvae. Hue, luminance (lightness), contrast, light transmission, chlorophyll content, photosynthetic activity and water content of the leaves were compared between herbivore-damaged and control trees. The leaves of herbivore-damaged trees had a decreased chlorophyll *a* concentration, increased contrast and they reflected more longer wavelengths. However, these changes are likely not obvious to birds. In contrast to our expectation, there were only minor differences in conspicuousness of larvae against the leaves of damaged trees, which may be very subtle to predator vision. Nevertheless, according to visual models, larvae should be easily detectable to birds from both herbivore-damaged and control trees.

Keywords: trophic interactions, avian vision model, background matching, herbivory, camouflage

## Significance statement

Herbivory affects photosynthetic machinery and light reflectance of leaves, and may thus provide visual foraging cues to birds, although it is not known how these changes appear to birds. We also hypothesised that the changes in leaves may reduce the camouflage of the herbivore. After applying herbivore-treatment and using the avian vision models, we found that the leaves of herbivore-damage may cause the leaves to appear to birds with higher contrast and greener or a more yellowish colour than control leaves. In addition, although the herbivore was visible to birds, it was slightly less conspicuous when on damaged trees, indicating that the herbivore can be adapted to changes in the food plant. Our results indicate that herbivory causes changes visual properties of leaves, but these changes are likely not obvious to birds.

## Introduction

One potential way for plants to decrease damage from herbivory is to attract the natural enemies of herbivores, such as predators or parasitoids. The ‘cry for help’ hypothesis suggests that predators of the herbivores can detect herbivore-damaged trees from a distance and are attracted by herbivore-mediated systemic changes in plants. Thus, the effect of herbivory on the metabolism of the plant may expose herbivores to predation, because the plant likely benefits from providing cues about the presence of herbivores. Correspondingly, several studies have shown that some parasitic wasps and predatory mites utilize volatile organic compounds (VOCs) released from herbivore-damaged plants as foraging cues (Turlings et al. 1990; Takabayashi and Dicke 1996; Kessler and Baldwin 2001; Hilker et al. 2002; Birkett et al. 2003; van Wijk et al. 2008). Most studies have focused on this VOC-based attraction of predators, but herbivory could also change the photosynthetic activity and/or light reflectance of even intact parts of the plant (Oleksyn et al. 1998; Zangerl et al. 2002; Retuerto et al. 2004; Mäntylä et al. 2008a, b, 2017; Amo et al. 2013; Hussain et al. 2014). Therefore, changes in photosynthesis and/or other visual properties of the plant may provide visual foraging cues to predators.

Interestingly, several behavioural experiments have shown that insectivorous birds are able to detect herbivore-damaged trees even when the herbivores and the damage have been removed from the birds’ sight (Mäntylä et al. 2004, 2008a, b, 2014, 2017; Amo et al. 2013, 2016), indicating that birds use cues related to the systemic responses by plants. Although the question whether birds can visually distinguish between herbivore-damaged and intact trees has not been tested with an avian vision model, it is possible that birds are able to see the changes in plants. In fact, there is some evidence supporting the hypothesis that birds use changes in light reflectance as visual foraging cues (Mäntylä et al. 2008b), while other evidence support the use of VOCs as olfactory foraging cues (Mäntylä et al. 2008a, 2014, 2017; Amo et al. 2013). However, visual and olfactory cues were not separated in studies by Mäntylä et al. (2008a, 2014, 2017) and therefore the results may be explained by other correlated factors and not by VOC cues alone. In the only study where these cues were offered separately to birds, only the olfactory cue hypothesis was supported (Amo et al. 2013). However, Koski et al. (2015) did not find support for this hypothesis. All in all, only a few studies have investigated the effect of herbivory on plant-mediated visual cues to birds (Mäntylä et al. 2008b, 2017; Amo et al. 2013), and to our knowledge changes in the visual properties of leaves have not been quantified. Hence, it is not known how herbivory affects the spectral composition or light transmission of the leaves, and how these changes in plants are viewed by birds. Consequently, the question remains whether birds utilize visual or olfactory cues or combination of these cues when detecting herbivore-damaged trees.

We hypothesise that in order to act as visual ‘cry for help’ signal from plants to birds, herbivory should not only decrease the chlorophyll content or photosynthetic activity of leaves (Zangler 2002; Mäntylä et al. 2008a), but also affect their colour, luminance (perceived lightness), achromatic contrast and/or light transmission so that these changes are visible to birds. Furthermore, we present and test a new ‘reduction in camouflage’ hypothesis, stating that the herbivore-mediated changes in photosynthetic pigments or light reflectance of leaves can indirectly decrease the efficacy of the camouflage and thus reveal the herbivore to predators. This, in turn, could direct the insect herbivores to change their colour during their development in order to improve their camouflage through background matching and countershading. In background matching, an animal attempts to match the colour, luminance, and pattern

of the background (Thayer 1909; Endler 1978, 1984; Edmunds and Dewhirst 1994; Stevens 2007; Merilaita and Stevens 2011; Merilaita and Dimitrova 2014). Many lepidopteran larvae have green colouration that matches to the colour of the photosynthetic tissue of the host plant (e.g. Edmunds 1990). Countershading pigmentation, in turn decreases the brightness contrast between the highly illuminated (darker pigmentation) and less illuminated (lighter pigmentation) sides of the animal, making it appear optically flat and hiding self-generated shadow (Thayer 1896; Cott 1940; Kiltie 1988; Ramachandran 1988; Rowland et al. 2007, 2008; Allen et al. 2012). However, herbivore-mediated systemic changes in plant defence may cause chromatic mismatches between the herbivore and plant due to changes in chlorophyll content and leaf colour, while changes in luminance and light transmission of leaves may increase their achromatic difference. We assume that changes in both chromatic and achromatic matching between herbivore and plant are important because insectivorous birds are likely to use both of these cues when detecting cryptic prey from the background (Stobbe et al. 2009). Furthermore, low chlorophyll content may change the light transmission of the leaves of herbivore-damaged trees so that the predator can see the larvae both from above and below the leaves, making the larvae especially exposed to predation. Thus, in this scenario, we would expect the larvae to be more detectable when on herbivore-damaged trees.

It is important to note that the ‘cry for help’ and ‘reduction in camouflage’ hypotheses are not necessary mutually exclusive and could function simultaneously. For example, from the plant’s point of view, herbivore-mediated changes in the visual properties of the leaves could act both as “long distance cues” (i.e. birds orient to trees where they likely find prey), and as “short distance cues” (i.e. to aid the detection of the prey among leaves by reducing the herbivore camouflage). However, the new ‘reduction in camouflage’ hypothesis is different from ‘cry for help’ hypothesis because it does not require the bird to be attracted to plants due to visual cues, but changes in the visual properties of leaves can make the prey easier for the predator to see when it is already foraging on the plant and searching for the prey. ‘Reduction in camouflage’ could thus work independently from ‘cry for help’, although they can also share plant physiological mechanisms, if the cry for help is mediated by vision.

To study the effects of herbivory on the visual properties of leaves, we manipulated herbivore-damage in silver birches (*Betula pendula*) by adding leaf-chewing autumnal moth larvae (*Epirrita autumnata*) on the experimental trees. We applied an avian vision model of blue tit (*Cyanistes caeruleus*) to investigate whether herbivory causes optical changes in the leaves that may be visible to birds and whether these changes make the larvae potentially more detectable. In addition to using visual models, chlorophyll concentration, fluorescence induction (i.e. indicators of photosynthesis), light transmission, and water content of the intact leaves of herbivore-damaged and undamaged control trees were measured.

## Methods

### Study system

Silver birch is one of the most common deciduous trees in Finland and it is a host to several insect species (Koponen 1983; Heimonen et al. 2015), including the autumnal moth. Because the autumnal moth is a common generalist (Silvonen et al. 2014), its camouflage may provide intermediate matching to several backgrounds, instead of close matching to a single host plant species (Merilaita et al. 1999; Houston et al. 2007), although to human eyes the larvae closely resemble the leaf colour of many deciduous species

(Fig. 1). The blue tit was chosen as a model predator, not only because it is a natural predator of the autumnal moth, but also because it is a common insectivorous species that forages on leaves and branches of deciduous trees (Moreno 1981), and may use herbivore-mediated changes in visual properties of leaves as foraging cues (Mäntylä et al. 2008b). In addition, blue tits have also been used in numerous studies as a representative model of an avian visual system for similar bird species (e.g. Siddiqi et al. 2004; Cazetta et al. 2009; Spottiswoode and Stevens 2011; Thurman and Seymoure 2015; Mäntylä et al. 2017).

### Field experiment

The field experiment was conducted during May and June 2009 at the Botanical Garden of the University of Turku (60°26' N, 22°10' E), on the island of Ruissalo in Turku, south-west Finland. The experimental trees were micropropagated silver birch clones (Jokinen et al. 1991) that were planted on the grounds of the garden as three-year-old saplings in 1994. The mother trees of the clones were from southern and central Finland and they had been selected because of their good growth and quality (Poteri et al. 2001). In early May 2009, 16 pairs of trees representing eight clones (although not in equal shares, see below) were chosen for the experiment. The trees in each pair were alike in genotype and in phenotype (i.e. similar height and structure), and did not have obvious herbivore damage. All the trees grew close (< 10 m) to each other on the edge of the tree stand, and one side of each tree was exposed to direct sunlight. For practical reasons, only birches that had branches in the lower part of the trunk were selected. One tree of each pair was randomly assigned to the herbivore treatment and the other tree was used as a control. We had four replicate pairs of two clones, two replicate pairs of two clones and one pair of four clones. Clone-specific responses to the herbivore damage are beyond the scope of this study but were controlled for in most statistical analyses.

Three mesh bags (approximately 80 × 35 cm, mesh 0.3 mm) were placed on three branches in the lower part of each experimental tree at the time of bud break in 5 May. Half of the trees were assigned to herbivore treatment group, where 20 laboratory-hatched second instar larvae were placed into each mesh bag of the trees (altogether 60 larvae per tree). The rest of the trees were left as control group, where the mesh bags in each tree were left empty. In herbivore treatment group, the larvae were allowed to feed until June 1st, and then both the larvae and bags were removed. The estimated loss of leaf area varied between 30 and 100 % in the larval bags (mean ± SD was 79 ± 13 %), confirming the strength of the herbivore treatment.

### Image data

To test for visual differences between the leaves of herbivore-damaged trees compared to control trees, and whether autumnal moth larvae are more easily distinguishable to birds on leaves of herbivore-damaged trees, we used calibrated digital photographs (see Stevens et al. 2007; Troscianko and Stevens 2015). Photographs were taken from five undamaged leaves per tree (collected from similar light conditions), and from 80 of 4<sup>th</sup> or 5<sup>th</sup> instar larvae (reared on herbivore-damaged trees, see above) with Fujifilm Finepix S3 Pro UVIR digital camera. The larvae were anesthetized with CO<sub>2</sub> before photographing them against a grey plotting paper, and the leaves from each tree were taken randomly from intact branches outside the mesh bags. The leaves were photographed within half an hour after sampling and the same leaves were later used in chlorophyll content analysis (see below). Since most

birds are able to see some degree of UV light (approximately 300–400 nm), the camera was equipped with a UV transmitting lens (Coastal Optical Systems) and an image sensor that was sensitive to UV and 'human-visible light' (spanning approximately 400–700 nm). In addition, photographs were taken under a light bulb emitting visible and UV wavelengths (Arcadia Fluorescent Bird Compact Lamp). Two photographs were taken from each leaf and larva: a 'human-visible light' photo taken with a filter blocking UV and infrared (Baader UV/IR Cut; 400–700 nm transmittance), and a second photo "UV photo" taken with UV-pass filter (Baader U; 310–400 nm transmittance) transmitting only in the UV region. A grey standard (Labsphere Spectralon diffuse reflectance standard), reflecting 50 % of all light across the avian visual spectrum, was included in every photo.

Regular photographs are optimised for human viewing and they usually have nonlinear pixel value responses to changes in light intensity, which under- or overestimates the true spectrum of light reflected from the object. Consequently, it is not possible to objectively specify colour of the object by measuring RGB values from a point of a regular photograph, especially when using a non-human visual system (Stevens et al. 2007, 2009; Troscianko and Stevens 2015). In order to reliably measure colour and brightness and to convert images to animal visual system, photographs need to be linearized to measure the spectral sensitivities of the camera images as well as standardized to control changes in light conditions (Lovell et al. 2005; Stevens et al. 2007, 2009; Pike 2011; Troscianko and Stevens 2015). Therefore, we used the Image Calibration and Analysis Toolbox (Troscianko and Stevens 2015) in ImageJ (Schneider et al. 2012) to combine the human visible images with corresponding UV images and to make these combined images comparable by normalizing and linearizing them against the grey standard. From the normalized and linearized leaf-images, a ca. 3 mm × 3 mm patch (i.e. region of interest, hereafter referred as ROI) was selected for image analysis using the plotting paper in the image as a scale. It was not possible to use blinded method for ROI sampling for the colouration of the birch leaves, because each photograph had individual labelling identifying the tree from which the leaf was collected. Furthermore, variation in hue and brightness of the leaves (including the UV-component visible to birds but not for humans) between control and experimental trees cannot be distinguished by the human eye. To minimize observer bias, the ROI was the same size, and on the left side of the central vein of the leaf if possible. However, to avoid large veins and areas where light reflected brightly back (specular reflectance) from the wax layer of leaves, the ROI was always taken only from an area where the leaf appeared dark green in the "human vision" photograph (in visible wavelengths) or dark in a UV photograph. If the leaf image had a strong shiny reflectance, it was discarded and ROI selection was not made. Thus, the total number of leaf images used was 122, but the number of images per tree varied from 1 to 5 (mean  $3.8 \pm \text{SD } 1.0$  per tree). However, total number of leaf images was equal for control and herbivore-damaged trees ( $n = 61$  for each treatment). In the case of the larval images, some of the images were discarded due to movement of a larva during photographing (larvae started to become active), causing misalignment between the human visible and UV photograph. In total 45 larval images were used (mean  $2.7 \pm \text{SD } 1.4$  per herbivore-damaged tree). The ROI (2–3 segments long) was taken from the dorsal side of the lateral line of each larva. This section of the larvae is green with thin yellow stripes in "human-visible" photograph, and appears dark in UV photograph (see Fig. 1 for a regular photograph demonstrating coloration of a larva). The yellow lateral line of a larva was never included in the ROI, but the thinner yellow lines could not be excluded.

## Avian vision model

An avian vision model was used to test how herbivore-mediated changes in leaves may be detected by insectivorous birds and whether these changes affect the predicted ability of birds to differentiate larvae on leaves. Four single cones are likely used in colour vision (long wavelength sensitive, i.e. LWS; medium wavelength sensitive, i.e. MWS; short wavelength sensitive, i.e. SWS; and ultraviolet sensitive, i.e. UVS) (Cuthill 2006). Cone spectral sensitivity is further tuned by oil droplets, and many birds likely see far more shades of colour than humans (Endler and Mielke 2005; Cuthill 2006). The fifth cone type includes double cones that are likely used for luminance-based tasks (Maier and Bowmaker 1993; Vorobyev and Osorio 1998; Vorobyev et al. 1998; Osorio and Vorobyev 2005), such as edge detection and detection of small objects. Although there is a broad similarity across species in their visual pigments, there is variation among species in the visual pigment sensitivity. Much of the variation appears to come from sensitivity to UV- or violet wavelengths: while still being able to detect UV-light, violet sensitive species (e.g. corvids, raptors) are relatively less sensitive to UV light compared to UV-sensitive species (e.g. many higher passerines) (Hart 2001; Hart and Vorobyev 2005; Endler and Milke 2005 and references therein).

To convert the ROIs from the normalized and linearized images of leaves and larvae to predicted photoreceptor responses of single and double cone types of a blue tit (Hart et al. 2000; Hart 2001; Troscianko and Stevens 2015), a mapping function in the Image Calibration and Analysis Toolbox was used. This converts the image data from camera colour space to the predicted cone response data of a focal visual system. Previous studies have shown that this mapping approach is highly accurate compared to reflectance-based calculations of predicted cone responses, and in fact very likely also better accounts for ambient illumination, angle of measurement, and larger measurement areas of the object/scene of interest (see data in Stevens and Cuthill 2006; Pike 2011; Troscianko and Stevens 2015). The vision model converts the ROIs to cone-catch data, i.e. to the relative photon catches of blue tit's four single cones, as well as to luminance values based on the double cone sensitivity. In order to describe the colour properties of the leaves, we calculated a measure of hue based on deriving a colour channel that best explained variation in colour, following a range of previous studies (e.g. Komdeur et al. 2005; Evans et al. 2010; Spottiswoode and Stevens 2011; Stevens et al. 2014). This is broadly based on the idea that colour perception in animals stems from antagonistic opponent colour channels that are frequently represented by a ratio (e.g. in humans, the red-green colour channel is  $LWS / MWS$ ) (Lovell et al. 2005). Although there is some evidence in birds for specific opponent colour channels, it is at present unknown which opponent pathways exist (Osorio et al. 1999). Our aim here was not to mimic a real or even putative channel in avian vision, but rather to provide calculation of hue based on an informed colour channel that best explained variation in colour in an intuitive and interpretable manner. We therefore followed past methods (e.g. Spottiswoode and Stevens 2011) and used principal component analysis on a covariance matrix of the standardised single cone catch values (i.e. values of each cone were transformed to proportions to remove variation in brightness) of the leaves to inform which colour channel should be used to encode hue in avian vision. The extracted principal component scores (Appendix, Table A1), provided a calculation of hue of leaves by using the formula  $(MWS+LWS) / (UVS+SWS)$ . An increase in score values above 1 means that leaves have shift to longer wavelengths, i.e. are more green or yellow,



whereas values smaller than one suggest a shift towards more blue-UV colours. These hue values were used in later statistical analyses.

In addition, colour and luminance discrimination models (Vorobyev and Osorio 1998) were conducted on cone-catch data from leaves and larvae to test the how well blue tits may discriminate (1) between the leaves of control and herbivore-damaged silver birch trees and (2) between autumnal moth larvae and leaves. The model assumes that receptor noise limits visual discrimination (Vorobyev and Osorio 1998; Vorobyev et al. 1998). To answer the first question, colour and luminance were analysed by comparing all control leaves against all defoliated leaves. To answer the latter question, colour and luminance analyses were conducted by comparing every leaf against every larva. Four single cones were used for the colour discrimination model, whereas the luminance discrimination model was based on the double cones (Siddiqi et al. 2004). In the colour discrimination model, a Weber fraction of 0.05 was used for the most abundant cone type, and the relative proportions of cone types in the blue tit retina (longwave = 2.08, mediumwave = 2.17, shortwave = 1.84, and ultraviolet sensitive = 1.00). A Weber fraction 0.05 was also used for modelling luminance discrimination using the double cones (Siddiqi et al. 2004; Sandre et al. 2010).

The discrimination model uses units of ‘just noticeable differences’ (hereafter, JNDs) where values  $<1$  to  $3$  indicate that the two colours are likely indistinguishable under optimal light conditions and values  $>3$  indicate that two objects are likely discriminable (Siddiqi et al. 2004). Technically, the discrimination model only predicts, based on JNDs, whether the two objects are discriminable and difference between JND values that are already above threshold may not further indicate if and by how much more objects are to further tell apart (Kelber and Osorio 2010; Kemp et al. 2015). However, some recent studies do indicate that increasing JND values above threshold can still indicate better discriminability (Cazetta et al. 2009; Nokelainen et al. 2012; Renoult et al. 2015; Fleishman et al. 2016). For example, Cazetta et al. (2009) showed that higher chromatic contrast (higher JND values) above the threshold of detection between artificial fruits and their background corresponded to higher probability of these fruits being detected by frugivorous birds. In addition, a behavioural test by Fleishman et al. (2016) with brown anole (*Anolis sagrei*) showed that the probability of discriminating two colours increased with JND values, indicating that JND values may provide a good estimate of relative conspicuousness of colours even beyond the discrimination threshold. Consequently, we cautiously interpret increasing JND values above threshold as potentially indicating increasing discriminability, although experiments similar to Fleishman et al. (2016) are needed for insectivorous birds in the future.

### Light transmission of leaves

One or two leaves per tree (28 trees in total) were placed on top of a light source for transillumination and the leaves were photographed from above (camera details described above). The same grey standard as used in colour photos was also included in every photo and the photos were converted to black and white images. The mean grey value of each leaf and the grey standard were recorded in ImageJ program.

### Chlorophyll concentrations, fluorescence and leaf water content

The same leaves that were used in avian vision model were used to determine concentrations of chlorophylls *a* and *b* with the method of Inskeep and Bloom (1985). For chlorophyll determination, a 0.21

cm<sup>2</sup> disk was cut from each leaf and incubated overnight in the dark in *N,N*-dimethylformamide. Absorbance was then measured at 646, 664 and 750 nm and the concentrations of chlorophylls *a* and *b* were calculated using the extinction coefficients of Inskeep and Bloom (1985).

Chlorophyll *a* fluorescence induction curves were measured from the same leaves with a PAM-101 fluorometer (Heinz Walz, Effeltrich, Germany). The leaves were dark adapted for at least 30 minutes before the measurements. First, the  $F_0$  value was measured using the dim measuring beam, then an illumination protocol was started, with 2 s measuring beam alone, 2 s red light, PFD (photosynthetic photon flux density)  $50 \mu\text{mol m}^{-2}\text{s}^{-1}$ , 1 s measuring beam alone, 1 s far red light, and a 2 s saturating pulse, PFD  $5500 \mu\text{mol m}^{-2}\text{s}^{-1}$ , 1 s measuring beam alone, and 1 s far red light.

The results were used to calculate  $F_v/F_M$ , or  $(F_M - F_0)/F_M$ , where  $F_M$  stands for the maximum chlorophyll fluorescence value obtained at the top of the saturating pulse, and it indicates the maximum yield of the photochemical reaction of Photosystem II. Photosystem II, in turn, is a “weak point” of the photosynthesis apparatus and therefore  $F_v/F_M$  is an appropriate indicator of the general health and lack of significant stress of the photosynthesis machinery of the plant (Krause and Weis 1991; Tyystjärvi 2008; Takahashi and Murata 2008).

In addition to the chlorophyll content, leaf water content was measured because herbivore-induced changes in water content may change visual characteristics of the tree, by affecting, for example the turgidity or optical properties of the leaves. Samples of 15–20 intact leaves from each tree were weighed on 29 May, then freeze-dried for 48 hours and reweighed. The relative water content (i.e. mass of water divided by fresh weight) of the leaves was calculated.

In chlorophyll concentrations, fluorescence and leaf water content measurements randomization of the samples was not needed, because the measurements cannot be affected by observer bias.

## Statistical analysis

### *Visual properties of the leaves*

To test the effect of herbivore treatment on visual properties of leaves, hue and contrast values of leaves were set as dependent variables in linear mixed models (LMM, for normal error distribution) and GLMM (generalized linear mixed model for lognormal error distribution) analyses, respectively. Both of these variables were calculated from the predicted photoreceptor responses of the blue tit. To allow comparison between leaves, standardised contrast for each leaf ( $n = 61$  for control and  $n = 61$  for leaves from herbivore-damaged trees) was calculated by the formula: contrast = luminance SD / luminance mean (Troscianko et al. 2016). In all analyses, treatment was set as an independent variable. In hue analysis, clone and individual tree nested within clone were set as random effects, while in the contrast analysis, individual tree was set as a random effect.

To test whether blue tits are potentially able to discriminate the leaves of herbivore-damaged trees from leaves of control tree based on colour or luminance, each control leaf was tested against each leaf from herbivore-damaged trees in the JND colour and luminance discrimination models. Based on these pairwise comparisons, the average values for the colour and luminance discriminations between treatments were calculated.

### *Difference in visual properties between larvae and leaves*

A LMM analysis with normal error distribution was conducted for the difference in standardized contrast (calculated from the predicted photoreceptor response values of the blue tit) between larvae and leaves (dependent variable). The difference was calculated by subtracting the standardized contrast of a larva from the contrast of a leaf [i.e. (luminance SD/luminance mean of a larva) – (luminance SD/luminance mean of a leaf)] so that each larva (n = 45) was compared against every leaf (n = 122). Treatment was set as an independent variable, while a tree and individual larva nested within tree were set as random effects.

To test the ability of birds to discriminate larvae from leaves, each leaf was compared individually to each larva in colour and luminance JND discrimination models. Based on these pairwise comparisons, average JND colour and luminance discrimination values were calculated for each leaf and used as dependent variables in separate LMMs with normal error distribution. Treatment was set as independent variable. Clone, and individual tree nested within clone were set as random effects.

#### Light transmission of leaves

The standardised light transmission of leaves was calculated by subtracting the average grey value of a tree from the mean value of a standard and using this difference as a dependent variable in a LMM analysis with normal error distribution. Treatment was set as an independent variable, while clone was set as a random effect.

#### *Chlorophyll concentrations, fluorescence and leaf water content*

The concentrations of chlorophyll *a* and *b*, total chlorophyll concentration (*a* + *b*),  $F_v/F_m$ -value from leaf fluorescence measurements and an average leaf water content of a tree were all used as dependent variables in separate LMMs with normal distribution. Treatment (control or herbivore-damaged trees) was set as an independent variable in all analyses. Clone was set as a random effect in leaf water content analyses while in the other analyses, clone and an individual tree nested within clone were set as random effects. The latter random effect accounted for the fact that the leaves of a tree used in the analyses were non-independent replicates of the same tree. Model assumptions (normality and homoscedasticity) were checked by inspection of the residuals.

Associations between the average hue of a tree (calculated from hue values of individual leaves) and the average chlorophyll concentration of a tree (calculated from chlorophyll *a* and *b* values of individual leaves) were calculated by Pearson's product-moment correlation coefficients. The correlation analyses were conducted separately for both herbivore treatment categories to overcome the impact of the main treatment.

All linear mixed models were conducted using the GLIMMIX procedure of the SAS statistical software, version 9.4 and the Kenward and Roger method (the latest version, Kenward and Roger 2009) was used to compute denominator degrees of freedom. For each analysed response, model-derived, estimated marginal means with their 95 % confidence intervals are given for the fixed effects in the text or Figs of the Results section.

## Results

## Differences in the visual properties of leaves

The mean ( $\pm$  SE) colour JND discrimination between the leaves of control and herbivore-damaged trees was  $2.97 \pm 0.14$  and for the luminance JND discrimination  $3.20 \pm 0.15$ , indicating that the ability of blue tits to discriminate between treatment levels based on colour or luminance is poor, though still high enough to potentially allow discrimination.

However, hue values (i.e. description of colour channels that best explained variation in colour) were significantly higher in leaves of herbivore-damaged trees, indicating a shift to longer wavelengths ( $F_{1, 24.41} = 4.37, P = 0.05$ ) (Fig. 2). In addition, leaves of herbivore-damaged [0.082 (95 % CI: 0.075 to 0.090)] trees had significantly higher ( $F_{1, 26.6} = 5.02, P = 0.03$ ) contrast compared to control trees [0.071 (95 % CI: 0.065 to 0.078)]. The treatment did not affect the light transmission of leaves ( $F_{1, 18.36} = 0.02, P = 0.88$ ).

## Differences in contrast, colour, and luminance between larvae and leaves

The colour JND discrimination was significantly greater ( $F_{1, 21.64} = 4.27, P = 0.05$ ) when larvae were on control leaves [mean 13.5 (95 % CI: 12.9 to 14.0)] compared to defoliated leaves [mean 12.8 (95 % CI: 12.3 to 13.4)] (Fig. 3a), but there was no difference in luminance JND discrimination ( $F_{1, 21.81} = 2.44, P = 0.13$ ) between treatment levels [control mean 6.1 (95 % CI: 5.1 to 7.1), defoliated mean 5.3 (95 % CI: 4.3 to 6.3)] when larvae were tested against leaves (Fig. 3b). However, note that with the colour results the differences in JNDs between control and herbivore damaged leaves are both well above threshold and differ on average in less than 1.00 JND. Therefore, it is questionable whether there are perceptually detectable effects between treatments despite the statistical difference therein.

In addition, the difference in contrast between larvae and leaves was larger ( $F_{1, 29.84} = 4.95, P = 0.03$ ) when larvae were tested against the leaves of the control trees compared to when larvae was tested against the leaves of the herbivore-damaged trees (Fig. 3c).

## Chlorophyll concentration, fluorescence measurements and leaf water content

The concentration of chlorophyll *a* was lower in the intact leaves of herbivore-damaged trees compared to control trees ( $F_{1, 24.58} = 6.29, P = 0.02$ ) (Fig. 4). There was no obvious difference in concentration of chlorophyll *b* ( $F_{1, 24.45} = 1.61, P = 0.22$ ) between control [4.7 (95 % CI: 4.4 to 5.1)  $\mu\text{g per cm}^2$  of leaf] and herbivore-damaged trees [4.6 (95 % CI: 4.2 to 4.9)  $\mu\text{g per cm}^2$  of leaf]. The lower chlorophyll *a* concentration in herbivore-damaged trees led to significant differences also in total chlorophyll (*a* + *b*) concentration ( $F_{1, 24.77} = 5.59, P = 0.03$ ) between control [26.6 (95 % CI: 24.8 to 28.4)] and herbivore-damaged trees [24.8 (95 % CI: 23.0 to 26.6)  $\mu\text{g per cm}^2$  of leaf].

Concentrations of chlorophyll *a* correlated negatively with the hue of the leaves both in the control ( $r = -0.652, P < 0.01, n = 16$ ) and the herbivore-damaged trees ( $r = -0.587, P = 0.02, n = 16$ ). A non-significant indication of negative relationship was also obtained for measurements of chlorophyll *b* and hue ( $r = -0.402, P = 0.12$  and  $r = -0.477, P = 0.06$ ) for control and herbivore-damaged trees, respectively.

The  $F_V/F_M$ -value did not differ ( $F_{1, 24.9} = 0.36, P = 0.55$ ) between the control [0.84 (95 % CI: 0.82 to 0.86)] and herbivore-damaged [0.83 (95 % CI: 0.81 to 0.85)] trees, indicating that the herbivore

damage to part of the tree did not cause significant stress to the photosynthetic machinery of the leaves. No obvious differences were detected in patterns of fluorescence induction curves between the control and damaged trees (data not shown).

Leaf water content percentage did not differ between the control [67.9 (95 % CI: 67.0 to 68.8)] and herbivore-damaged trees [68.7 (95 % CI: 67.8 to 69.6)] ( $F_{1,28.3} = 0.86$ ,  $P = 0.36$ ).

## Discussion

Natural enemies of herbivores may use chemical and visual information from plants to locate their prey (e.g. Schaefer and Ruxton 2011), which likely benefits both plants and predators. From the herbivore's perspective, changes in the photosynthetic activity and/or light reflectance of plants, caused by herbivore feeding (Oleksyn et al. 1998; Zangerl et al. 2002; Retuerto et al. 2004; Mäntylä et al. 2008a, b; Amo et al. 2013; Hussain et al. 2014), may increase the vulnerability of the herbivore to predators, as insectivorous birds may use these changes as visual foraging cues (Mäntylä et al. 2004, 2008b; but see Amo et al. 2013). Furthermore, these changes in the plant may increase the conspicuousness of the herbivore to predators also by reducing the background matching of the herbivore against leaves. We found that the trees responded systemically to herbivore damage: herbivory shifted the hue of leaves to longer wavelengths (Fig. 2) making the intact leaves from herbivore-damaged trees to potentially appear “greener or more yellowish” to birds (blue tits) compared to leaves from the control trees. In addition, the leaves of herbivore-damaged trees had higher achromatic contrast (luminance SD/luminance mean) and significantly lower concentration of chlorophyll *a*. Because both contrast and hue values were received by converting the reflectance values to predicted photoreceptor responses of the blue tit, these results suggest that birds may use changes both in hue and contrast of leaves as visual cues when detecting herbivore-damaged trees. However, the low colour and luminance JND discrimination values suggest that the changes in the visual properties of leaves between control and herbivore-damaged trees are not obvious and may instead offer limited detection to insectivorous birds. Furthermore, given that the differences in colour and luminance discrimination values among treatment levels were not substantial, further behavioural ecology work is needed to determine if birds can and do respond to these differences.

In contrast to our ‘reduction in camouflage’ hypothesis, changes in the visual properties of leaves did not increase the conspicuousness of the herbivore. Rather, because colour JND values (Fig. 3a) and difference in contrast (Fig. 3c) were higher when larvae were tested against control leaves, autumnal moth larvae may be less conspicuous on the damaged trees compared to undamaged control trees. Note also that the differences in JNDs between control and herbivore-damaged trees were very small and both well above threshold, suggesting that any perceptible differences would be minor, if detectable at all. There was no significant difference between treatment levels in how well birds discriminate larvae against leaves based on luminance (Fig. 3b), even though the JND values were quantitatively higher when the larvae were on control leaves. However, this finding should be treated with considerable caution because, as with colour, the JND values in both treatments were well above threshold for detection and the JND differences for larval discriminability against control and herbivore-damaged trees were very small. The high colour JND values between larvae and leaves could be explained by thin yellow stripes of the larvae that could not be excluded in the ROI selection (see Fig. 1 and ‘Image data’ in methods). In addition, the ROI selection from leaves could contribute to the colour JND differences because it was

done from the darkest part of the leaves to avoid areas where light reflected brightly back from the wax layer. The colour and luminance JND values between larvae and leaves nevertheless suggest that even though the colour of the larvae closely resembles the colour of leaves to human eyes (Fig. 1), larvae should be detectable for birds on leaves on both control and herbivore-damaged trees. This is in accordance with findings by Stobbe et al. (2009) that insectivorous birds can use both chromatic and achromatic cues in detection of cryptic prey. The conspicuousness of the larvae may also be related to the fact that autumnal moth is a generalist, and therefore likely adapted to intermediately resemble several backgrounds (host plants) rather than being well-matched to a single or a few host plants (Merilaita et al. 1999; Houston et al. 2007). The camouflage of the herbivore may not only be affected by diet, but also be dependent on the plastic response of a larva to cues of the appearance of the host plant (Noor et al. 2008; Sandre et al. 2013). In our study system, however, the closer matching between the autumnal moth larvae and leaves of the damaged trees was likely due to diet as the larvae were used as defoliators. It would be interesting to test the plasticity of the camouflage of autumnal moth to avian predators by using several food plants to see whether the effect of visual appearance of the food plant can exceed the effect of diet also in non-polyphenic larvae. Studies, such as Fleishman et al. (2016) combining behavioural experiments and visual system modelling, are needed to test how birds actually respond to herbivore-mediated changes in leaves and how these changes affect the camouflage of the herbivore.

Changes in hue and contrast in the leaves of herbivore-damaged trees may be explained by changes in leaf chemistry. We found that the concentration of chlorophyll *a* was not only significantly lower in the leaves of herbivore-damaged trees compared to control trees (Fig. 4), but it was also strongly negatively correlated with hue. There was also a trend in negative correlation between chlorophyll *b* and hue in herbivore-damaged trees. The lower concentration of chlorophyll *a* also resulted in lower total concentration of chlorophyll in herbivore-damaged trees. However, contrary to previous studies (Zangerl et al. 2002; Mäntylä et al. 2008a) and our hypothesis, this decrease did not significantly affect the  $F_v/F_M$  value although herbivory affected the hue, contrast, and chlorophyll concentration. In addition, there was no difference in the light transmission of leaves, despite that transmittance spectra can be different from reflected spectra (Gates 1980). Evidence from tomato (*Solanum lycopersicum*) suggests that herbivory-induced physiological changes in plants may depend on plant and herbivore species and on the physiological state of the plant. For example defoliation of tomato by *Manduca sexta* larvae caused loss of chlorophyll in young but not in old leaves (Korpita et al. 2014), and the specialist herbivore *Helicoverpa zea* caused more alterations in metabolites than the generalist *M. sexta* (Steinbrenner et al. 2011).

To conclude, our results indicate that herbivory may influence on interactions between plants, insect herbivores and their avian predators by causing systemic changes in plants, thus supporting recent behavioural studies that have shown responses of birds to herbivore-mediated systemic changes in plants (Mäntylä et al. 2004, 2008a, b, 2014, 2017; Amo et al. 2013, 2016). We did not find support for the ‘reduction in camouflage’ hypothesis as the larvae are likely easily detectable for birds against the leaves of both control and herbivore-damaged trees. However, although perceptible differences are likely minor between treatments, the herbivore may be slightly less conspicuous to birds when on damaged plants on some circumstances or to some individuals. It is possible that intraspecific phenotypic and genotypic variation often observed in cryptic coloration (e.g. Sandre et al. 2007; Canfield et al. 2009) could be

maintained both via changes in predator's behaviour (e.g. search image) (e.g. Greenwood 1989; Bond and Kamil 2006) as well as from food plants' behalf. Finally, variation in the conspicuousness against the background could also facilitate the evolution of costly secondary defences for a cryptic prey (see e.g. Endler and Mappes 2004; Lindstedt et al. 2011) as after being detected, secondary defences could increase the probability to survive. This could also offer a stepping stone for the evolution of aposematic coloration for a chemically defended prey.

#### Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

#### Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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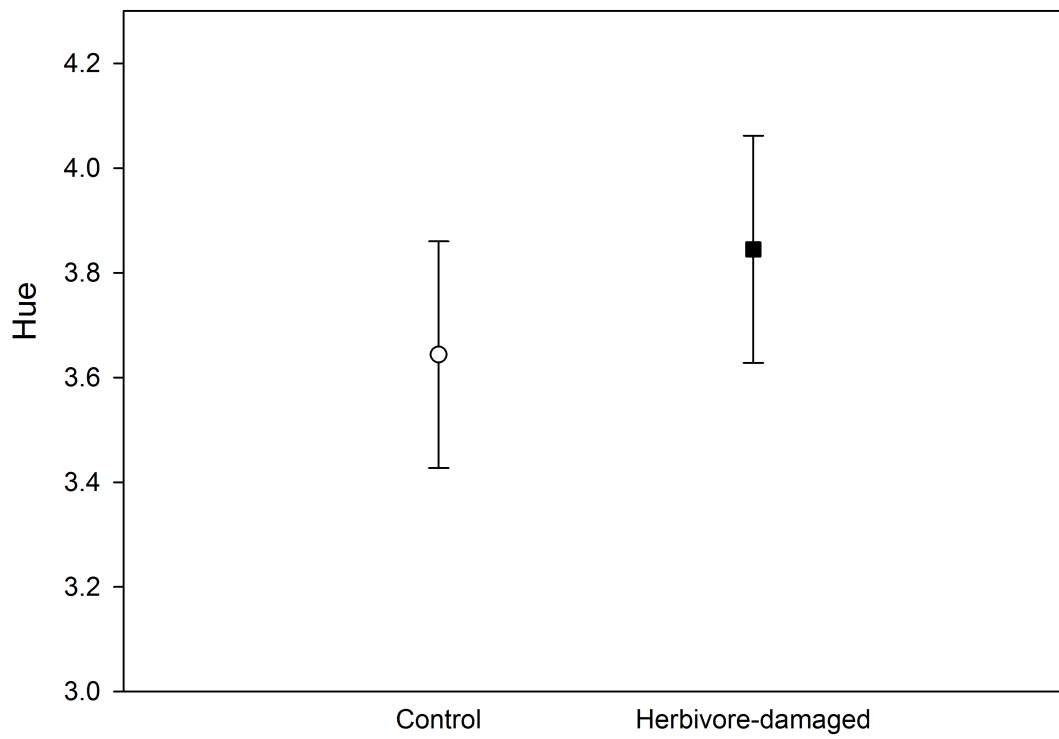
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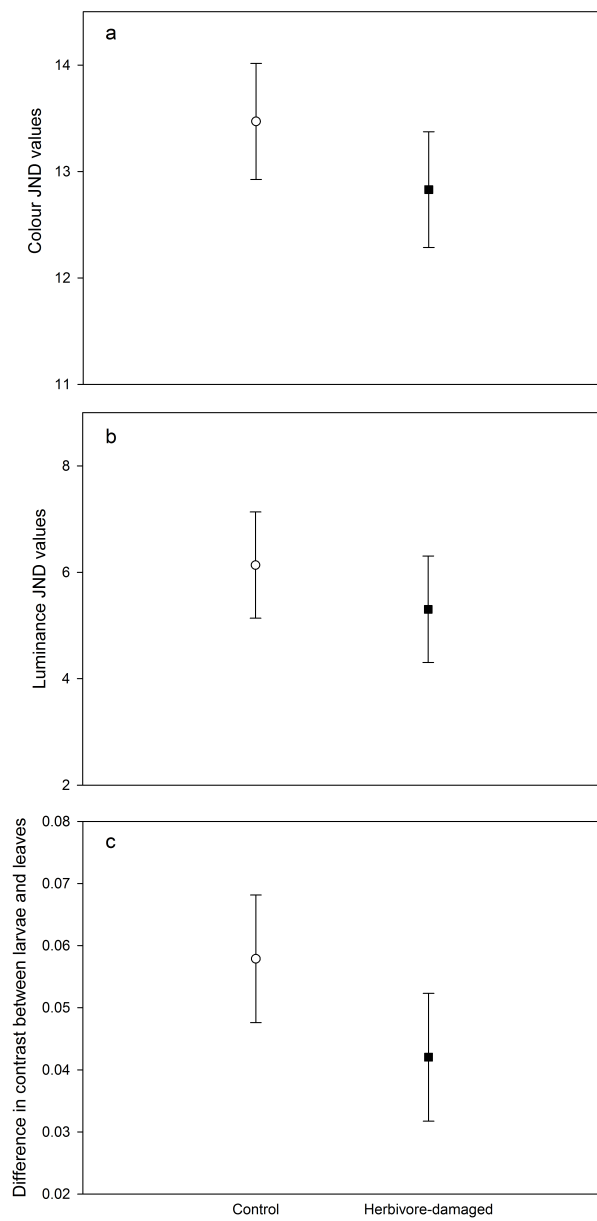
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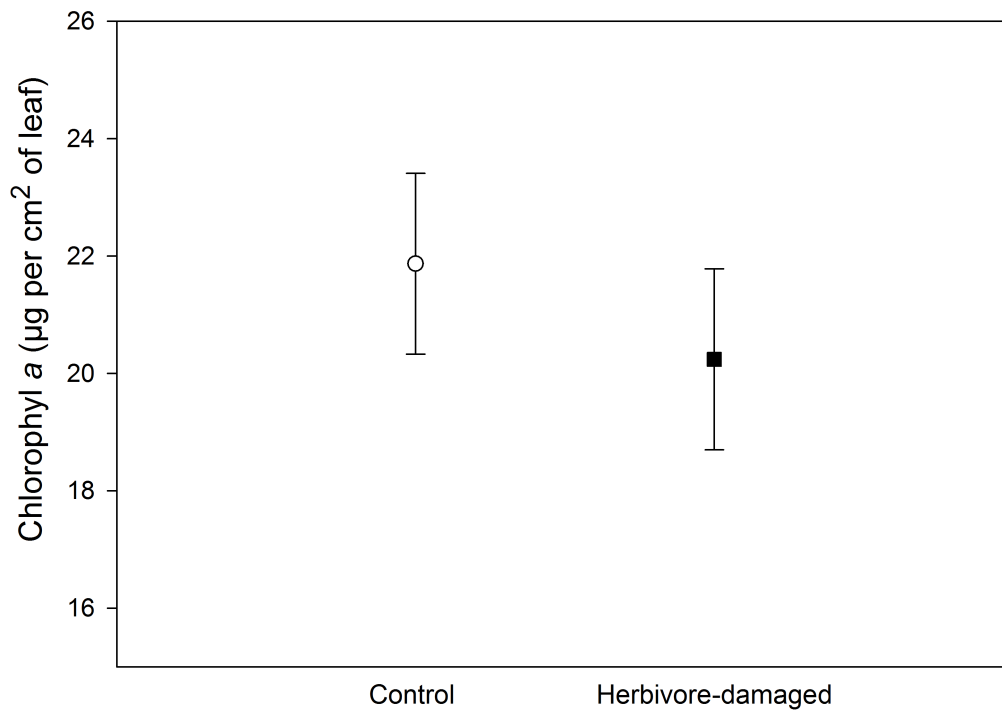
**Fig. 1** Image of an autumnal moth larva on a birch leaf as a demonstration of colouration of the species in a regular photograph (photograph by Tero Klemola). A patch of 2–3 segments long from the dorsal side of the lateral line was selected for the image analyses from every normalized and linearized photograph of larvae.



**Fig. 2** Mean (95 % CI) hue values of control and intact leaves of herbivore-damaged trees ( $P = 0.05$ )



**Fig. 3** Conspicuousness of autumnal moth larvae to blue tit against leaves of control and herbivore-damaged silver birches based on mean (95 % CI) values of **a**) colour ( $P = 0.05$ ), **b**) luminance ( $P = 0.13$ ), and **c**) difference in contrast ( $P = 0.03$ )



**Fig. 4** Mean (95 % CI) chlorophyll *a* concentrations sampled from control (16 trees, 61 leaves in total) or intact leaves of herbivore-damaged (16 trees, 62 leaves in total) silver birches ( $P = 0.02$ )



## Appendix

Table A1 Result from principal component scores explaining variation in leaf colour using uv, short wave, medium wave and long wave sensitive cone values of blue tit

Variable	Principal component 1 (eigenvalue 3.160, explained 79 % of the variation)	Principal component 2 (eigenvalue 0.614 explained 15.3 % of the variation)
UVS mean	0.513	-0.383
SWS mean	0.508	0.422
MWS mean	-0.496	-0.555
LWS mean	-0.483	0.606