

Red and yellow pigments in autumn leaves are associated with higher nitrogen resorption

A comment on Peña-Novas and Marchetti 2021 (<https://doi.org/10.1111/jeb.13903>)

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A reply to Pena-Novas and Archetti (2021b) “A test of the photoprotection hypothesis for the evolution of autumn colours: Chlorophyll resorption, not anthocyanin production, is correlated with nitrogen translocation”.

The prevalence of autumn colours in many temperate regions of the world has generated many hypotheses on the putative adaptive value of this phenomenon over the years (Archetti, 2009). Yellow leaves are caused by the breakdown of the green chlorophyll that reveals the yellow carotenoid pigments present throughout the year, whereas red anthocyanin pigments are generated *de novo* in the fall to cause red leaves (reviewed in Archetti et al., 2009; Renner & Zohner, 2019).

Two categories of explanations have been put forward to explain the putative adaptive value of autumn colours: protection against abiotic factors and animal–plant interactions (reviewed in Archetti et al., 2009). Amongst the many hypotheses that fall into either of these categories, two have been evaluated with more scrutiny. The coevolution hypothesis (Archetti, 2000) suggests that the red colour acts as a warning signal for insects such as aphids that migrate to trees in the fall, thereby preventing the plants from herbivory damages in the following spring. Alternatively, the photoprotection hypothesis (Hoch et al., 2001) proposes that the anthocyanins and carotenoids protect the leaves from photo-oxidative stress under cool temperatures and intense light (Esteban et al., 2015; Gould et al., 2018; Havaux & Kloppstech, 2001) that occur in the fall with the breakdown of chlorophyll. The pigments could act directly by acting as a sunscreen or indirectly by neutralizing reactive oxygen species and other photoreactive metabolites, and in doing so they would allow for a better resorption of nutrients such as nitrogen and phosphorous (Archetti et al., 2009; Renner & Zohner, 2019). If both hypotheses have received considerable interest in the past, their respective merit has been the subject of a renewed debate recently (Agati et al., 2021; Pena-Novas & Archetti, 2020a, 2020b, 2021a, 2021b; Renner & Zohner, 2019, 2020).

Pena-Novas and Archetti (2021b) recently evaluated one prediction of the photoprotection hypothesis, which is that leaves of plants with autumn colours should have a better resorption of nitrogen in the fall. The authors analysed a data set of 55 species with a comparative method and observed that although the raw data suggested greater resorption of nitrogen in species with both red and yellow leaves compared to species with green leaves, only the species with yellow leaves had a significantly higher nitrogen resorption than species with green leaves when taking the phylogeny of species into account. This result lead them to conclude that red anthocyanin pigments in leaves are not associated with nitrogen resorption, providing evidence against the photoprotection hypothesis.

After reanalysing the data of Pena-Novas and Archetti (2021b), the non-significant result of higher resorption in red leaves appears to be incorrect and caused by the little power of the statistical approach used by the authors. When analysing multiple species, observations cannot be considered to be independent because closely related species may react similarly due to traits or behaviour that evolved in their shared common ancestors. To avoid such as bias, Pena-Novas and Archetti (2021b) analysed pairs of species that were selected in such a way that the branches of the phylogeny are never considered more than once when tracing lines between species along the phylogeny (Maddison, 2000; Read & Nee, 1995). Whilst this species-pairs approach indeed results in an analysis that is unbiased relative to the phylogeny (Felsenstein, 2004), it also results in the exclusion of many species from the data set and reduces the power of the statistical analysis, a point that was acknowledged by the authors (Pena-Novas & Archetti, 2021b). A better approach to account for the phylogenetic non-independence of species in a data set is to model the phylogenetic correlation that can be present in the residuals of a linear model using phylogenetic generalized least squares (PGLS; Grafen, 1989). Such an approach has the advantage to use all the information present in the original data set.

I thus reanalysed the data of Pena-Novas and Archetti (2021b) using a PGLS approach. I used the data presented in the paper and the Zanne et al. (2014) phylogenetic tree that was used by the authors. Species not present in the tree were replaced by a congeneric species or by the closest species available (see Appendix S1). I then fitted a PGLS model using the 'glS' function of the nlme R package (Pinheiro et al., 2020). I used the Pagel (1999) phylogenetic correlation structure from the ape R package ape (Paradis & Schliep, 2019) and estimated the lambda parameter with the model using restricted maximum likelihood (REML). The lambda parameter normally varies between 0 and 1, that is, from none to perfect phylogenetic correlation. The use of the Pagel correlation structure thus allows the residuals of the model to be adjusted according to the adequate level of phylogenetic correlation, which is important for the analysis to be unbiased (Revell, 2010). Resorption values were squared to ensure the normality of the residuals. The effect of the leaf coloration on nitrogen resorption was tested with *F*-statistics using the 'ANOVA' function and post hoc Tukey tests were performed to test significance between the categories using the 'glht' function of the multcomp R package (Hothorn et al., 2008). The scripts used for the analyses are presented in Appendix S1.

The value of the lambda parameter fitted in the PGLS model was 0.39 and this model was significantly better than models with no phylogenetic correlation or with perfect phylogenetic correlation in the residuals (see Appendix S1). This result suggests that there was phylogenetic correlation in the residuals of the model but that there were also additional sources of error. The PGLS model suggests that colour variation strongly affected resorption of nitrogen (*F*-value = 7.116, *p* = 0.0019; Figure 1). Post hoc tests indicated that the resorption of nitrogen, which varies from 0 (no resorption) to 1 (complete resorption), was significantly different between species with red and green leaves ($\text{resorption}_{\text{red}}^2 - \text{resorption}_{\text{green}}^2 = 0.195$, std. error = 0.054, *p* = 0.0009) and between species with yellow and green leaves

($\text{resorption}_{\text{yellow}}^2 - \text{resorption}_{\text{green}}^2 = 0.162$, std. error = 0.049, *p* = 0.0026). In contrast, the comparison in resorption between species with red and yellow leaves was not significantly different than 0 ($\text{resorption}_{\text{red}}^2 - \text{resorption}_{\text{yellow}}^2 = 0.033$, std. error = 0.041, *p* = 0.6977). I reached the same conclusions when resorption was estimated by using the summer nitrogen content from the previous year and when species that were not present in the phylogeny were dropped from the analysis (see Appendix S1).

In the light of these results, I conclude that the resorption of nitrogen in the fall is greater in species with red or yellow leaves compared to species with green leaves in this sample of 55 species. This result supports the photoprotection hypothesis *sensu lato* (e.g. Renner & Zohner, 2019), because Pena-Novas and Archetti (2021b) chose to centre their photoprotection hypothesis only on red pigments that are produced in the fall (Pena-Novas & Archetti, 2021b). Yet, given that carotenoids also protect leaves from photo-oxidative stress (Young, 1991; Havaux & Kloppstech, 2001), both types of pigments should allow for better resorption of nitrogen. However, the fact that the different types of pigments were not quantified in this experiment limits the interpretation of the results and further research is necessary to better understand the processes involved in nitrogen resorption for leaves of different colours in autumn. This reanalysis does not imply that red leaves cannot also be involved in animal-plant interactions in some species (Archetti et al., 2009; Pena-Novas & Archetti, 2020a) and ultimately a combination of causes may be needed for explaining autumn colours. But the higher nitrogen resorption observed in species with red leaves compared to species with green leaves adds to other lines of evidence (Agati et al., 2021; Gould et al., 2018; Renner & Zohner, 2019) suggesting that anthocyanins have a photoprotective effect.

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CONFLICT OF INTEREST

The author reports no conflict of interest.

AUTHORS CONTRIBUTIONS

SJ performed the analysis and wrote the paper.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13961>.

OPEN RESEARCH BADGES



This article has earned an Open Data, for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.6084/m9.figshare.16862032.v1>.

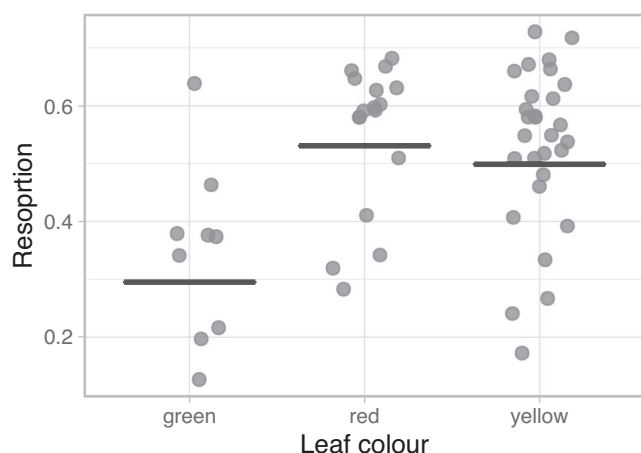


FIGURE 1 Nitrogen resorption for species with different leaf colours in autumn. The points represent the raw values per species and the horizontal bars the fitted means obtained with the PGLS regression

DATA AVAILABILITY STATEMENT

Scripts and data used to perform the analyses presented in this study were deposited in FigShare (<https://doi.org/10.6084/m9.figshare.16862032>).

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SUPPORTING INFORMATION

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