Letters

Reply to Lavi & Sapir (2015): floral colour and pollinator-mediated selection in Oncocyclus irises (Iridaceae)

In a recent research article published in *New Phytologist*, Lavi & Sapir (2015) investigated the adaptive significance of the large and dark flowers of irises belonging to the section Oncocyclus (genus *Iris*, family *Iridaceae*). These plants are strictly endemic to dry, Mediterranean-type climates, particularly to the semi-desert areas of the Middle East, Turkey and the Caucasus (Mathew, 1989). Using a selection gradients approach, Lavi & Sapir (2015) statistically tested the significance of flower size, stem length and floral colour on plant fitness in two Oncocyclus species (*Iris atropurpurea* Baker and *I. haynei* Baker). The authors used a standard approach by comparing the seed set of open-pollinated flowers to flowers receiving supplementary hand pollination to control for the effect of pollen limitation on plant fitness, thereby discriminating between pollinator-mediated selection on pheno-typic traits and other sources of selection.

Regression techniques have been used since the early 1980s to estimate the direction and magnitude of selection (Lande & Arnold, 1983), and it is now clear that measures of long-run selection at higher taxonomical levels (here, a group of *Iris* species) require a more thorough understanding of the selection processes, for example, by attempting to disentangle the role of environmental drivers (climate, fires, floods, etc.) on the strength of selection on floral traits (Rausher, 1992). In their recent work, Lavi & Sapir (2015, p. 369) used a single site (albeit the largest known) for each study species to conclude that 'phenotypic color variation in these irises is neutral'.

Countless authors have discussed the role of floral colours in floral biology and pollinator behaviour. Since the advent of modern spectrophotometry techniques and progress in uncovering the electrophysiological bases of insect vision, it has become clear that 'subjective human judgements of flower colour may be inaccurate and irrelevant to the ecology and vision of the flower's pollinators' (Arnold *et al.*, 2010, p. 1). In their article Lavi & Sapir (2015) measured the concentration of a single pigment (anthocyanin) in outer petal extracts of the irises by spectrophotometry at a single wavelength (530 nm) under laboratory conditions as a rough proxy for flower colour. The authors then interpreted the lack of correlation between anthocyanin concentration and female repro-ductive success as evidence for the absence of pollinator-mediated selection on floral colours in the Oncocyclus irises. By restricting their analytical approach to the quantification of anthocyanin concentration, Lavi & Sapir (2015) failed to take into account other important aspects of floral colour that drive changes in pollinator behaviour, such as the presence of co-pigments or other pigments (that influence floral hue, see e.g. Hopkins & Rausher, 2012; Sobel & Streisfeld, 2013) or other surface effects (such as epidermal cell shape and structure, see e.g. Papiorek *et al.*, 2014). Integrating knowledge of insect vision, including the patterns of photoreceptor excitation in trichromatic bees (the pollinators of these irises), as well as the effect of background colour on visual discrimination capabilities of different floral colours by bees (Arnold *et al.*, 2010) and results from other recent studies would have been beneficial to the discussion of the adaptive value of floral colours as perceived by the pollinators. Several colour space models such as the bee hexagon (Chittka, 1992) have been developed over the past two decades to depict how the spectral reflectance of flowers whose colour appear distinct to a human can look similar to a pollinator, and *vice versa*. These models (Chittka & Raine, 2006), have the advantages of first, allowing predictions on the bee-subjective appearance of object colours relative to their environmental background and second, in presenting this information in a quantitative graphical format. This approach is essential to interpret the perception of floral reflectance in its ecological context using photoreceptor sensitivities of specific pollinators, and was not used in the article by Lavi & Sapir (2015).

Lavi & Sapir's (2015) approach to the study of pollinator-mediated selection on floral colours is in contrast with our recent studies, where we have used a range of state-of-the-art methods for the analysis of floral colours and scents from a pollinator perspective and in a phylogenetic context (with reconstructions of ancestral pollination strategies). Indeed, our recent results on the role of floral colours and scents in the parallel evolution of shelter-mimicking flowers in the *Orchidaceae* (genera *Ophrys* and *Serapias*), and in the *Iridaceae* (section Oncocyclus) clearly demonstrate that virtually all extant shelter-mimicking plant species share the same suite of morphological traits, floral scent compounds and spectral patterns (such as 'colour') as adaptations to selectively attract their shared narrow taxonomical guild of specialized pollinators, primarily sheltering male bees (Vereecken *et al.*, 2012). Further-more, we also showed that the flowers of *Iris atropurpurea* in particular (a species investigated by Lavi & Sapir, 2015) is likely to have evolved by pollinator-mediated selection acting *primarily* on floral colours to mimic large achromatic (i.e. 'bee-black') protective shelters (Vereecken *et al.*, 2013). Finally, our *in situ* experiments on pollinator preferences also demonstrated that pollinator visits in the Oncocyclus irises are *not* motivated by an increased morning floral heat reward in tunnels facing the rising sun (Vereecken *et al.*, 2013). This is also supported by a series of studies on the thermoregulatory abilities of wild bees illustrating that a 2 to 4°C increase of heat within flowers might be regarded as meaningless in light of the very high body warm-up rates of most medium-to-large vernal solitary bees recorded as pollinators of the Oncocyclus irises (Stone &

Willmer, 1989; Heinrich, 1993; Willmer & Stone, 2004). Lavi & Sapir (2015) have not touched upon this research, and in their article they focus only upon 'morning floral heat' as a reward for pollinators (see their first sentence of the Discussion section, 'Pollinators of the Oncocyclus irises are attracted to the heat absorbed in the dark-colored flowers in the morning (Sapir *et al.*, 2006)'), a theory that is not statistically supported by any direct evidence in these irises (only indirect, correlative evidence with small sample sizes was provided; Vereecken *et al.*, 2013).

Finally, Lavi & Sapir (2015) note in their methods section that: 'These [*Iris*] species are completely self-incompatible, and maternal fitness (fruit and seed production) depends entirely on the night-sheltering pollinators (Sapir *et al.*, 2005; Watts *et al.*, 2013)'. However, Watts *et al.* (2013, p. 395) note explicitly that: '[. . .] Honey bees were found to be frequent diurnal visitors; they removed large quantities of pollen and were as effective as male sheltering bees at pollinating this species'. Our personal field observations during 2010–2011 suggest that the Netanya Iris Reserve where Lavi & Sapir (2015) conducted their study on *Iris atropurpurea* was not devoid of honey bees, which could have bearing on the interpretation of the results presented by Lavi & Sapir (2015).

We regret that Lavi & Sapir (2015) failed to build on the ever-growing body of scientific knowledge on the quantification of selection gradients driving the evolution of floral traits, and that they did not use the mainstream analytical tools to study floral evolution (here, floral reflectance) from a pollinators' perspective. We think it would have been appropriate to discuss the most recent (and contradictory) evidence stemming from comparative phylogenetic studies on the evolution of floral traits (Vereecken *et al.*, 2012) and from *in situ* measurements of pollinator preferences for floral scents or colours using state of the art methods (Vereecken *et al.*, 2013).

In this Letter we highlight our recent work on a topic highly relevant to the work presented by Lavi & Sapir (2015) (dealing with the same issues, in the same species, in the same biogeographical region) in order to provide the scientific community with a balanced view of contemporary research on the pollination of Oncocyclus irises. We encourage the community to adopt the use of modern methodologies that allow the analysis of floral traits from the pollinators' perspective. Only in this way will new light be shed on floral evolution in these and other fascinating examples of plant–pollinator interactions.

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