— Opinion —

A GLOBAL REVIEW OF POLLINATION SYNDROMES: A RESPONSE TO OLLERTON ET AL. 2015

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Abstract—In a recent literature review, we demonstrated that the evolution of floral traits is driven by adaptation to the most effective pollinators. In a critique of this study, Ollerton et al. 2015 claimed there were apparent flaws with data collection, analyses and interpretation of results. We disagree since many of OLT´s observations and recommendations are subjective and overlook basic aspects of meta-analysis. Here, we address the main criticisms of Ollerton et al 2015.

Keywords: *Floral traits, plant evolution, plant reproduction, pollination syndromes*

We recently published the first systematic quantitative review of pollination syndromes to test the idea that the most effective pollinators of plants can be inferred from suites of floral traits (Rosas-Guerrero et al. 2014). Our results show that 1) convergent evolution of floral phenotypes is driven by adaptation to the most effective pollinator group; 2) the predictability of pollination syndromes is greater in pollinator-dependent species and in plants from tropical regions; and 3) many plant species also have secondary pollinators that often correspond to the ancestral pollinators documented in evolutionary studies.

In this issue, Ollerton et al. 2015 (henceforth OLT) claim that the meta-analysis by Rosas-Guerrero et al. 2014 (henceforth R-G) had apparent flaws in extracting and analysing data from the literature and problems with interpretation of results. We strongly disagree. Many of OLT´s observations and recommendations are subjective, including an incorrect approach at re-analysing our results, overlooking basic aspects of the theory and statistical grounds of meta-analysis. OLT also pinpoint individual studies as particular exceptions to the patterns found by R-G, failing to see that individual studies used in meta-analysis are members of a population of studies, each providing information on a given phenomenon or effect, and not isolated examples. Meta-analysis overcomes subjective judgement of particular effects by shifting the interpretation of biological importance from case studies and textbook examples to "the weight of the evidence" across all of the literature on a particular question (Koricheva & Gurevitch 2014). Below we discuss the main flaws of OLT's criticisms.

OLT criticize the selection of studies, measurements of effectiveness, and what they considered different types of "missing values", including an apparent phylogenetic bias in R-G's meta-analysis. As in any other systematic review, we provided a transparent protocol of literature search including details of strict inclusion and exclusion criteria so this synthesis can be replicated. OLT´s criticisms regarding search protocols are mostly subjective and in some cases erroneous. We did not score all eleven traits for each plant species because information on certain traits (such as odours) is unavailable for most angiosperm species, and only a subset of traits is enough to assign a given pollination syndrome. Syndrome assignment was conducted by a designated group of co-authors using all information available on floral traits based on literature, field measurements, and specialists. We intentionally excluded crops because we consider them to be under anthropogenic influence. We searched for studies that quantified the pollination efficiency of the entire pollinator assemblage under natural conditions, whether they tested the pollination syndrome hypothesis or not. The 43% of species

in R-G with only one pollinator functional group represent specialized pollination systems that are common in nature (Fenster et al. 2004), and not missing values of secondary pollinators, as claimed by OLT. We provide evidence that there were no statistical differences among the effectiveness measurements used in R-G (Fig 1b R-G). Contrary to OLT's claim, our keyword combination (see p. 369 of R-G) did find the papers of Stebbins (1970), Levin & Berube (1972) and Primack & Silander (1975), because we reviewed studies since 1900 (R-G). The studies mentioned by OLT, among many others, were not included due to their lack of quantitative pollinator efficiency data to calculate effect sizes. To assess how the exclusion of studies affected the overall results, we conducted several analyses of publication bias, including the calculation of a weighted fail-safe number (Jennions et al. 2013 and references therein), which indicated that at least 47,000 studies with null effects are needed to compromise our overall results (p. 342 R-G). A different kind of problem of any review is research bias (*sensu* Gurevitch & Hedges 1999), which arises from non-random sampling of the natural world by primary researchers, and therefore, reviewers cannot correct for it. However, as presented by R-G, an important contribution of any literature review is to identify gaps in the literature where more research is needed.

OLT argue that we "only" included 18.7% of angiosperm families in our review. They claim that universal validation of syndromes requires a sample representing all angiosperms. Such an assertion denies the paradigme of statistical inference. Phylogenetically independent meta-analysis has been recently developed (Lajeunesse 2009) to account for the nested hierarchical structure associated to the shared phylogenetic history among species in the effect size calculations. Phylogenetically independent meta-analysis provides more powerful estimations (resolving non-independence problems) and allows detecting the relative influence of phylogeny in the overall results. By finding identical results between traditional and phylogenetic meta-analyses, R-G revealed a complete lack of phylogenetic bias. OLT did suggest interesting comparisons regarding the presence or absence of multiple secondary pollinators. While considered briefly in R-G, we have expanded on these and related ideas in a recent publication by Ashworth et al. (2015).

OLT disagree with our estimated weighted overall effect and its variance, expressed with bias-corrected 95% confidence intervals (CI's) around the overall mean or summary effect. They argue that graphical representation of results in meta-analysis can easily (and unintentionally) be misleading, and claim that Fig. 1a in R-G produces a "visual effect", which suggests that d values are clustered around the positive mean, so that the variance estimate for the overall mean is wrong. OLT also use Table S2 in R-G to count the null and negative effects of individual species. This approach is called vote counting, and it is a seriously flawed statistical technique for research synthesis (Borenstein et al. 2009; Koricheva et al. 2013), which is one of the reasons meta-analysis prevails in current ecological research syntheses. Based on such reasoning, OLT recommend that variation among species "would be better illustrated by showing ±

1.96 standard deviations of the actual d values", and offer "a new" standard deviation computed straightforward from all effect sizes from Table S2, as if these effects were raw primary data. By doing this, they ignore the way summary effects are calculated in meta-analysis, which typically incorporates the two sources of variation present in any ecological meta-data assuming random effects models: variation within study (i.e., within-study error, Vd) and true variation among effect sizes (i.e., between-study variance, tau (τ)). Consequently, it is not a matter of "visual effects" on whether CI's are larger or smaller around a mean value, it is a matter of proper calculation. Meta-analysis accounts for unequal precision of effect sizes among studies, achieved by weighting each study by the inverse of its variance ($1/Vd$). Thus, meta-analysis offers an improved control of type II error rates (Arnqvist & Wooster 1995), as the power to detect an overall effect increases by the accumulation of evidence across many studies, regardless of their individual P-value (Koricheva et al. 2013). In fact, contrary to the vote counting logic, meta-analysis can allow the detection of a significant overall effect (i.e., CI's non-overlapping zero) even when individual studies included in a synthesis do not show statistically significant results (Arnqvist & Wooster 1995, Koricheva et al. 2013).

Finally, OLT claimed that the first test of traditional pollination syndromes at a global scale was conducted by Ollerton et al. (2009, henceforth OLT09), who found contrasting results to R-G. However, these two studies are not comparable because, among other reasons, OLT09 did not properly quantify the effectiveness of each floral visitor in the pollinator assemblage. It is no surprise that our results contrast with those of OLT09, who sampled vegetation ($N = 482$ morphospecies) in three temperate and three tropical sites and observed frequency of pollinators for approximately half of these species. Moreover, OLT09 identified to the species level only 87% of temperate and 22% of tropical plant species, and recorded pollinators for only 50% of the identified plant species ($N = 92$ temperate spp, $N = 33$ tropical spp.). Furthermore, an evaluation of the technique used in OLT09 to assign syndromes is needed, since it resulted in clear mis-assignment of syndromes to some species, which in our opinion systematically resulted in disagreement between predicted syndromes and observed pollinators. Unfortunately, the limited sampling of species with proper taxonomic identification precludes replication and testing of OLT09, because it is impossible to re-sample unidentified species. Additionally, insufficient nocturnal observations might explain the reported disagreement between assigned moth or bat pollination syndromes and observed floral visitors in OLT09. Tropical sites had very limited representation of the most diverse tropical ecosystems (e.g. tropical dry forest, tropical rainforest), life forms, and taxonomic groups (e.g. trees, epiphytes, orchids). Thus, many of OLT's criticisms to our study apply to their own study.

To date, R-G is the most comprehensive and global analysis of pollination syndromes and it has the advantage that further studies can be easily incorporated in further tests of hypotheses. Our results imply that floral syndromes represent examples of convergent evolution driven by the

most effective pollinator. Over the last 200 years, naturalists have described the astonishing floral variation in the angiosperms, and studies of pollinator-mediated selection have demonstrated that animals are important selective agents on floral traits (e.g., Harder and Johnson 2009). Nevertheless, intense human disturbance of natural habitats has caused disruptions in patterns of mutualistic interactions that may partly explain the presence of the diverse pollinator assemblages that are frequently found in pollination studies (Quesada et al. 2011). Beyond the pollination syndrome controversy we now face the challenge of understanding the evolution of mutualisms in the Anthropocene, which will most likely increase the presence of multiple secondary pollinators replacing primary pollinators because of human disturbance.

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