Peer Community In
Evolutionary Biology

Sometimes, sex is in the head

Juan Arroyo *based on peer reviews by 3 anonymous reviewers*

Rubén Torices, Ana Afonso, Arne A. Anderberg, José M. Gómez and Marcos Méndez (2019) Architectural traits constrain the evolution of unisexual flowers and sexual segregation within inflorescences: an interspecific approach. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/356147

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Plants display an amazing diversity of reproductive strategies with and without sex. This diversity is particularly remarkable in flowering plants, as highlighted by Charles Darwin, who wrote several botanical books scrutinizing plant reproduction. One particularly influential work concerned floral variation [1]. Darwin recognized that flowers may present different forms within a single population, with or without sex specialization. The number of species concerned is small, but they display recurrent patterns, which made it possible for Darwin to invoke natural and sexual selection to explain them. Most of early evolutionary theory on the evolution of reproductive strategies was developed in the first half of the 20th century and was based on animals. However, the pioneering work by David Lloyd from the 1970s onwards excited interest in the diversity of plant sexual strategies as models for testing adaptive hypotheses and predicting reproductive outcomes [2]. The sex specialization of individual flowers and plants has since become one of the favorite topics of evolutionary biologists. However, attention has focused mostly on cases related to sex differentiation (dioecy and associated conditions [3]). Separate unisexual flower types on the same plant (monoecy and related cases, rendering the plant functionally hermaphroditic) have been much less studied, apart from their possible role in the evolution of dioecy [4] or their association with particular modes of pollination [5]. Two specific non-mutually exclusive hypotheses on the evolution of separate sexes in flowers (dicliny) have been proposed, both anchored in Lloyd's views and Darwin's legacy, with selfing avoidance and optimal limited resource allocation. Intermediate sex separation, in which sex morphs have different combinations of unisexual and hermaphrodite flowers, has been crucial for testing these hypotheses through comparative analyses of optimal conditions in suggested transitions. Again, cases in which floral unisexuality does not lead to sex separation have been studied much less than dioecious plants, at both the microevolutionary and macroevolutionary levels. It is surprising that the increasing availability of plant phylogenies and powerful methods for testing evolutionary transitions and correlations have not led to more studies, even though the frequency of monoecy is probably highest among diclinous species (those with unisexual flowers in any distribution among plants within a population [6]). The study by Torices *et al.* [7] aims to fill this gap, offering a different perspective to that provided by Diggle & Miller [8] on the evolution of monoecious conditions. The authors use heads of a number of species of the sunflower family (Asteraceae) to test specifically the effect of resource limitation on the expression of sexual morphs within the head. They make use of the very particular and constant architecture of inflorescences in these species (the flower head or "capitulum") and the diversity of sexual conditions (hermaphrodite, gynomonoecious, monoecious) and their spatial pattern within the flower head in this plant family to develop an elegant means of testing this hypothesis. Their results are consistent with their expectations on the effect of resource limitation on the head, as determined by patterns of fruit size within the head, assuming that female fecundity is more strongly limited by resource availability than male function. The authors took on a huge challenge in choosing to study the largest plant family (about 25 thousand species). Their sample was limited to only about a hundred species, but species selection was very careful, to ensure that the range of sex conditions and the available phylogenetic information were adequately represented. The analytical methods are robust and cast no doubt on the reported results. However, I can't help but wonder what would happen if the antiselfing hypothesis was tested simultaneously. This would require self-incompatibility (SI) data for the species sample, as the presence of SI is usually invoked as a powerful antiselfing mechanism, rendering the unisexuality of flowers unnecessary. However, SI is variable and frequently lost in the sunflower family [9]. I also wonder to what extent the very specific architecture of flower heads imposes an idiosyncratic resource distribution that may have fixed these sexual systems in species and lineages of the family. Although not approached in this study, intraspecific variation seems to be low. It would be very interesting to use similar approaches in other plant groups in which inflorescence architecture is lax and resource distribution may differ. A whole-plant approach might be required, rather than investigations of single inflorescences as in this study. This study has no flaws, but instead paves the way for further testing of a long-standing dual hypothesis, probably with different outcomes in different ecological and evolutionary settings. In the end, sex is not only in the head.

References:

[1] Darwin, C. (1877). The different forms of flowers on plants of the same species. John Murray.

[2] Barrett, S. C. H., and Harder, L. D. (2006). David G. Lloyd and the evolution of floral biology: from natural history to strategic analysis. In L.D. Harder, L. D., and Barrett, S. C. H. (eds) Ecology and Evolution of Flowers. OUP, Oxford. Pp 1-21.

[3] Geber, M. A., Dawson, T. E., and Delph, L. F. (eds) (1999). Gender and sexual dimorphism in flowering plants. Springer, Berlin.

[4] Charlesworth, D. (1999). Theories of the evolution of dioecy. In Geber, M. A., Dawson T. E. and Delph L. F. (eds) (1999). Gender and sexual dimorphism in flowering plants. Springer, Berlin. Pp. 33-60.

[5] Friedman, J., and Barrett, S. C. (2008). A phylogenetic analysis of the evolution of wind pollination in the angiosperms. International Journal of Plant Sciences, 169(1), 49-58. doi: [10.1086/523365](`https://dx.doi.org/` 10.1086/523365)

[6] Renner, S. S. (2014). The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. American Journal of botany, 101(10), 1588-1596. doi: [10.3732/ajb.1400196](`https://dx.doi.org/` 10.3732/ajb.1400196)

[7] Torices, R., Afonso, A., Anderberg, A. A., Gómez, J. M., and Méndez, M. (2019). Architectural traits constrain the evolution of unisexual flowers and sexual segregation within inflorescences: an interspecific approach. bioRxiv, 356147, ver. 3 peer-reviewed and recommended by PCI Evol Biol. doi: [10.1101/356147](`https://dx.doi.org/` 10.1101/356147)

[8] Diggle, P. K., and Miller, J. S. (2013). Developmental plasticity, genetic assimilation, and the evolutionary diversification of sexual expression in Solanum. American journal of botany, 100(6), 1050-1060. doi: [10.3732/ajb.1200647](https://dx.doi.org/ 10.3732/ajb.1200647)

[9] Ferrer, M. M., and Good-Avila, S. V. (2007). Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. New Phytologist, 173(2), 401-414. doi: [10.1111/j.1469-8137.2006.01905.x](https://dx.doi.org/ 10.1111/j.1469-8137.2006.01905.x)

Reviews

Evaluation round #2

Reviewed by anonymous reviewer 3, 11 November 2018

Dear Editor,

This second version of the manuscript nicely addressed all my concerns. Although not all of my suggestions were followed, authors provided a careful argumentation. Thus I have no further comments for improvement of the manuscript. I believe it will represent a strong contribution to the field.

Sincerely,

JF

Evaluation round #1

DOI or URL of the preprint: **10.1101/356147**
Version of the preprint: 1

Authors' reply, 08 November 2018

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Decision by **Juan Arroyo**, posted 08 November 2018

Revise

Dear Dr. Torices,

As a member of PCI in Evolutionary Biology I was asked to manage your preprint in order to get it recommended in this platform

"Architectural traits constrain the evolution 1 of unisexual flowers and sexual segregation within inflorescences: an interspecific approach"

Three competent colleagues made insightful reviews from different, albeit complementary fields, and their comments are available to you. I consider, like reviewers, that the preprint has a high value to be recommended by PCI Evol Biol, but at the same time, I consider that many of the comments and concerns raised by the reviewers are worth to be taken into account and responded properly. These reviews, albeit anonymous, and your responses will be posted in PCI Evol Biol portal. Since the reviews are made from different perspectives, I strongly believe that considering them in your response and/or revised manuscript will greatly enhance its quality and the readership of the final preprint.

I also would add some specific minor comments from my own side:

1. Given the very particular nature of the inflorescence type in your study, I would consider including some

indication in the title. It might be adding "...in Asteraceae", "flower heads", "compact inflorescences", etc. As the authors mention in their Discussion, it remains to be proved what is the case in other kind of inflorescences, specially those being sparse.
2. Please, consider to do an extra review of the English, I found some typos (e.g. line 76 "later") and format of the reference list (some journal titles are no italisiced, similarly to some species names, among others). Reference by Funk et al. 2009 is published by IAPT?. Please, consider that a preprint is a publically available text and that PCI Evol Biol does not support proof correction.
3. Line 134. I have some doubt about the meaning of "phylogenetic gradient".
4. Lines 186-188. A sample of 97 species might be low or high enough depending on its representativeness. It would be important to add more information in Table S1, for example adding the subfamilies, and the rough distribution of species. For those readers non-familiar with systematics of Asteraceae, a supplementary figure with the position of the sampled species in the Asteraceae tree would be very useful. I also wonder why four genera were represented by more than one species (particularly Vernonia). Did you think that there were within genus variability?, but this is not discussed in the manuscript. Is the tree used including within genus variability?
5. Line 374. Although I like the arguments raised by the authors, I consider that there is a possibility to test if geitonogamy (i.e. selfing) avoidance (as stated by Harder & Barrett 1995 considering pollinator movements) is involved by correlating SI data (as from Ferrer et al. 20007, cited by the authors) with your data, if possible. Incidentally, do you have wind-pollinated species in your data set? this information could be also useful in your Table S1.

References cited:

Ferrer, M. M., and S. V Good-Avila. 2007. Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. New Phytol. 173:401–14.
Funk, V. A., A. Susanna, T. Stuessy, and R. J. Bayer, eds. 2009. Systematics, evolution, and biogeography of Compositae. International Association for Plant Taxonomy, Viena.
Harder, L. D., and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515.

Reviewed by anonymous reviewer 3, 01 July 2018

Dear Editor,

This study examine whether sexual specialization (evolution of unisexual flowers within inflorescences) is related to architectural constraints conditioning the availability of resources for fruit and seed production. Authors proposed that the architecture of the inflorescence affect the availability of resources for early and late flowers within inflorescences affecting the expression of sexual specialization. In turn this mechanism will result in higher sexual specialization among those species with stronger architectural constraints (larger difference between outer and inner fruits and seeds). The study used a comparative approach with a sample of 70 species of Asteraceae which have flowers grouped in capitula and present different levels of sexual dimorphism at the inflorescence level (from cosexual hermaphroditic flowers to monoecious unisexual flowers). Overall the manuscript is clearly written and easy to follow. Methods, and Results are nicely presented and support the main conclusions. I do have however, a couple of points for further improvement of the presentation and discussion of the results.

1. Lines 61-75: I believe it might be important to consider those selection pressures that can operate upon variation in fruit size within infrutescence. For instance, dimorphism and strong variation in seed size in desert Asteraceae has been recorded as a strategy to deal with unpredictable environmental conditions (see Venable studies with desert species). In the absence of a significant pattern related to sexual specialization this can be an alternative explanation.

2. Lines 76-78: The following two reasons relies on the expectation that resources for fruit production

are limited. Although this is a general expectation widely accepted, it would be useful to support this assumption with published evidence that added unlimited resources and still found positional effects on fruit size. This will indicate a true positional constraint. In addition, if differences in fruit size disappear under unlimited resources, then resource limitation will be the major constraint. Could you please provide more support for this central assumption of your work. This will add to your main conclusion posted at the end of the Abstract.

3. Lines 445-446: I would consider that modularity may increase within individual variance reducing the power to detect patterns at higher levels of organization.

4. Line 465: I would expect also that different inflorescence type may impose differences in the magnitude of architectural constraints and FSD.

Reviewed by anonymous reviewer 1, 09 July 2018

This study explores the idea that sexual specialization is driven by resource allocation and architectural constraints in angiosperms (i.e., that male-only flowers evolve as a response of having insufficient resources to develop seed). Generally, I found this study to outline a clever approach, with its use of herbarium specimens and phylogenetic comparisons. My criticisms are that: (1) I think they could have outlined the mechanism a bit better (i.e., it hinges on trade-offs in resource allocation in plants that make new resources throughout the growing season - many would argue that if the resources are replenished through photosynthesis, then a trade-off is unlikely to exist), and (2) at times, I struggled with parts of the methodology although I think all of that confusion could be ironed out with more information. I outline below where I felt more information or a more thorough treatment of alternative hypotheses is needed.

Line 122-124: Overall, I found the Introduction to be very well-written and a pleasure to read. Some of the sections could use more clarity however. E.g., here, I found it confusing that they state that a negative association between size and number could obscure patterns. It seems to diminish the approach. I suggest rewording to end on how you can deal with this confounding pattern.

Line 194: Flower density seems a bit of a problematic metric, being constructed from inflorescence size and number of flowers per inflorescence. I would need more convincing that it isn't confounded with flower size.

Line 199: Please provide data on how the fruiting heads of Asteraceae "usually" retain the size and structure of the inflorescence. Some of your conclusions hinge on this claim.

Line 204: What is "good" conservation status?

Line 204: "For each species, one specimen was selected" - how was this one specimen chosen? Was it randomized?

Line 212: descriptions of fruit size from the projection seems unnecessarily vague. I'm assuming you got a measurement of fruit area (please state this, if so).

Line 259: If was not clear to me why phylogenetic reduced major axis regressions were conducted with phyloRMA and also phylo.RMA

Line 262: There is a great deal of attention to fruit size in this paper yet it is odd that there is so little on flower size. Are flower size and fruit size not correlated? Small flowers will likely make small fruits which will diminish the size differences between early and late fruits I expect. Large flowers (will have large fruits) yet may be selected to increase pollinator visitation. These alternative hypotheses are explored later in the manuscript only in the sense of flower number and its effects on selfing rate. Flower density seems to be used as a type of proxy for flower size (with an implied trade off between size and number) but I didn't see this explicitly treated in any way (other than the mention that flower size and fruit size are roughly the same, with no data to back this up).

Line 313: I found the results interesting but thought that habitat and growing envrironment were likely confounding variables. The hypothesis being tested seems to operate only if you can imagine preemption of resources as the growing season comes to an end. Does it not likely matter where these species are growing (i.e., in environments with short or long growing seasons?). Therefore, I'm not sure that it's helpful that the species comes from different continents and biomes as stated earlier.

Reviewed by anonymous reviewer 2, 09 July 2018

Torices and collaborators in the manuscript entitled ”Architectural traits constrain the evolution of unisexual flowers and sexual segregation within inflorescences: an interspecific approach” test the hypothesis that inflorescence architecture may impose a constraint on resource availability for late flowers, which may potentially lead to different optima in floral sex allocation and unisexuality. They expect that inflorescence traits increasing the difference in resource availability between early and later flowers would be phylogenetically correlated with a higher level of sexual specialization. They have performed comparative analyses of inflorescence traits (inflorescence size, number of flowers and flower density) to test this hypothesis in the Asteraceae family (including different levels of sexual specialization: hermaphroditic, gynomonoecious and monoecious species). Their main results are that monoecious species had significantly denser inflorescences and species with denser inflorescences showed greater differences in the size of early and late fruits (this was used as a proxy of resource variation between flowers). Finally, they concluded that floral sexual specialization may be the consequence of different floral sex allocation optima driven by the sequential development of flowers that results in a persistent resource decline from earlier to later flowers.

The authors present an interesting case study to investigate. The topic of the paper is interesting and novel. In general the paper is well written, the analyses appear well executed and the main conclusions are well argued for. The authors provide substantial data and analyses for addressing the questions posed. The strengths of this manuscript include the well-developed motivating question and the ideal study group of organisms to test the hypothesis.

However, they are a number of major and minor issues that need to be justified (listed below). I will try to provide suggestions on how the manuscript could be substantially improved and how these issues could be handled.

1. Number of inflorescence per individual. This is an issue that needs to be at least discussed. I understand that probably there are species that will produce many inflorescences per individual and other species will produce one inflorescence per individual. I think the paper would gain if the authors discuss how it could affect their conclusions.
2. Sampling. The authors have highlighted the very high species richness of family Asteraceae. They have only included 100 species in this study. The authors should discuss how these species are distributed in the family. And how their very low sampling of the Asteraceae lineage could affect their conclusions.
3. Intraspecific variation. They have measured only ca. of one herbarium sample per species. I would like to know how the intraspecific variation has been estimated and whether or not it has been included in their phylogenetic comparative method analyses.
4. Authors have used Likelihood Ratio Test for model selection. AIC and other criteria have been proved to provide better results.
5. The phylogeny they have used was published in 2005 (over ten years ago) with a calibration from a paper from 2010. During the last decade it has been an exponential improvement of phylogenetic and divergence estimates methods. In the last years many new studies has been published with better estimates of divergence time in all branches of the tree of life, including the family Asteraceae (see Time Tree of Life here `http://www.timetree.org/`). I wonder whether or not the phylogeny and branch lengths estimates could be significantly improved.
6. I like the phylogenetic comparative methods approach that authors have used. But I think they could be significantly improved. They have used BM and OU models. However, they have only used an OU model with a single optimum. This model might be used with two, three, four, five, etc. optima. The data seem to suggest that the best models could have two or three optima. Including analyses with two and three optima OU models (one for each of the sexual specialization: hermaphroditic, gynomonoecious and monoecious species) would

significantly improve the manuscript.

This paper was the first to show this approach: Butler, M. A., & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. American Naturalist, 164(6), 683-695. doi:10.1086/426002. They are many examples in the literature using this several optima approach. See these three examples among many:
Nürk, N. M., Michling, F., & Linder, H. P. (2018). Are the radiations of temperate lineages in tropical alpine ecosystems pre-adapted? Global Ecology and Biogeography, 27(3), 334-345. doi:10.1111/geb.12699. Schmitz, L., & Higham, T. E. (2018). Non-uniform evolutionary response of gecko eye size to changes in diel activity patterns. Biology Letters, 14(5) doi:10.1098/rsbl.2018.0064. Zanne, A. E., Pearse, W. D., Cornwell, W. K., McGlinn, D. J., Wright, I. J., & Uyeda, J. C. (2018). Functional biogeography of angiosperms: Life at the extremes. New Phytologist, 218(4), 1697-1709. doi:10.1111/nph.15114.