Roddy et al. Hydraulic constraints of reproduction do not explain sexual dimorphism in the genus Leucadendron (Proteaceae)

This well-written manuscript reports morphological data, and data on stem hydraulic flux, from two species of the South African dioecious genus Leucadendron. The central aim of the paper is to assess the veracity or utility of the 'arguments' supposedly made in a paper by Harris and Pannell (Journal of Ecology, 2010) concerning a possible link between sexual dimorphism in branching architecture and the degree of serotiny in females (i.e., canopy seed storage) across species of the genus. The data presented are quite interesting and draw attention to important concepts and ideas concerning branching architecture and how to measure it, sexual dimorphism and how to measure it, allometric relations in plant morphology, and the concept of hydraulic efficiency in plants. It also raises questions concerning the potential differing costs of reproduction of males and females and the nature of tradeoff constraints. The manuscript is thus potentially conceptually rich.

Although I found the manuscript stimulating, I was also disappointed and frustrated by it. First, very little information was given in the manuscript concerning the sampling methods, yet these are critical to evaluating the strength of the claims being made. I am grateful to the first author, Adam Roddy, for clarifying certain points by email, and for sending me his dataset. Several aspects of what this clarification and the data reveal are important to highlight. First, the dataset is very small (a few individuals measured in each of two species) and does not measure up to our much larger study that the authors criticize in their manuscript (for comparison, we studied many more individuals in fully 49 species of the genus). Second, the individuals measured by Roddy et al. were not sampled in a non-random way. The reader should thus lack confidence that the dataset provides a representative view of the populations concerned. Third, the authors criticize a metric that we used to measure branching architecture. However, they used a method to estimate our metric that was inappropriate, especially for one of the two species sampled. Fourth, the authors imply throughout their paper that we were overly simplistic and narrowly focused in our interpretation of our data, but their depiction of our paper is a partisan caricature that does not acknowledge that our 'arguments' or 'predictions' were in fact explicitly speculative attempts to explain a surprising and novel discovery, and that, in discussing our speculation, we advanced other hypotheses and conceded that our ideas may not ultimately be sustainable. Roddy et al., however, imply that we were uncritically wedded to a particular interpretation of our data, which I just don't think is true. Thus, I think much of the weight of their manuscript amounts to criticism of a straw man. Finally, and I think most importantly, Roddy et al.'s manuscript suffers from a failure to recognise that species can be sexually dimorphic in some traits but not in others – or indeed in complex traits measured in one way but not, or differently, in another. They conflate this important fact with evidence that a particular metric must be erroneous if it fails to reveal a pattern that a very different metric finds, or vice versa. Most alarmingly in this context, the authors measure the relative 'accuracy' of the metrics they compare against a subjective standard based on what should be 'obvious' to the reader, or at least to them.

Below I rehearse these criticisms in detail by drawing attention to a number of passages in Roddy et al.'s manuscript that trouble me. They are listed in their order of appearance in the text.

Abstract, Background: Roddy et al. state: “Harris and Pannell (2010, Journal of Ecology 98:509-515) argued that these two traits–serotiny and sexual dimorphism–may be linked because the transpirational costs of maintaining seed cones for many years would select for increased female hydraulic efficiency, resulting in reduced branch ramification among females and greater sexual dimorphism. However, this argument assumed that shoot hydraulic efficiency is related to sexually dimorphic traits.” This slightly misrepresenting the text in the cited paper. In fact, Harris and Pannell found a clear association between serotiny and sexual dimorphism in Leucadendron, but, in their Discussion, they suggested that “Three nonmutually exclusive hypotheses may explain this association.” They went on to discuss the three hypotheses, including the one criticized by Roddy et al. However, Harris and Pannell did not ‘argue’ for this hypothesis, but stated, much more tentatively: “We speculate that increased costs in terms of water may select for thicker branches in serotinous females, and thus reduced ramification, compared with males, because thick branches are able to conduct water more efficiently.” In a subsequent paragraph, Harris and Pannell state: “However, it is also possible that […] the patterns in sexual dimorphism and serotiny in Leucadendron are being driven by some other factor.” This is quite different from _arguing_ single-mindedly for a particular cause of the association found. Roddy et al. should tone down their claims about what we wrote.

Abstract, Results: Roddy et al state: “We found that the metric of branch ramification used by Harris and Pannell (2010) to characterize sexual dimorphism does not conform to known biophysical scaling relationships”. Although not explicit, there is an implicit implication here that Harris and Pannell’s index was adopted because it conforms with biophysical scaling relationships; readers could be misled by this statement. Harris and Pannell adopted a particular metric for quantifying sexual dimorphism that they judged to be useful. One might like to argue that there are better metrics for sexual dimorphism in branching architecture than the one adopted by Harris and Pannell. However, there is no logic in choosing a metric that must conform to anything whatever. Any metric of sexual dimorphism is based on a comparison of measurements on males and females. Whether it has any bearing on biophysical, biochemical or other factors might be interesting, but it surely cannot be necessary.

Abstract, Discussion: Roddy et al state: “Instead, these results argue for a more nuanced view of plant physiology and morphology that acknowledges that physiological constraints may be satisfied by adjustments in multiple traits and that multiple agents of selection are likely responsible for the evolution of morphological traits.” More nuanced than what? The implication is that Harris and Pannell’s view was less nuanced, and that they did not acknowledge “that physiological constraints may be satisfied by adjustments in multiple traits and that multiple agents of selection are likely responsible for the evolution of morphological traits”. But this is a misrepresentation of Harris and Pannell’s paper. A quick reading of their Discussion reveals that they considered several different explanations for the association that their paper documents, and indeed it ends by suggesting that the patterns of sexual dimorphism in Leucadendron are likely attributable to the importance of selection on both male and female reproductive strategies, e.g., of pollination biology and resource allocation by males and females. Importantly, Harris and Pannell offer one indirect test of the idea that water relationships might have a bearing sexual dimorphism: they looked for an association with patterns of precipitation experienced by the sampled populations. They found no evidence for this association, so that their speculation about this point remained unsupported by their study. They considered reasons for finding no support, including “that the patterns in sexual dimorphism and serotiny in Leucadendron are being driven by some other factor”. Roddy et al. should represent the results and discussion in Harris and Pannell more accurately, avoiding an implication to naive readers that our Discussion may have been uncritical or one-sided.

Lines 72-78: Again, Roddy et al. claim that Harris and Pannell “argued” for a particular cause of sexual dimorphism in Leucadendron. See my comments on the Abstract. The authors should more accurately represent our text; we speculated on the possible implications of water relations for sexual dimorphism, among other hypotheses discussed. It would be fair and reasonable, and would take no more than one sentence, to explain to the reader that, for instance: “Harris and Pannell conducted a phylogenetically controlled comparative analysis of 49 species of Leaucadendron and found a clear association between a measure of sexual dimorphism in branching architecture and the degree of serotiny in females. In interpreting their results, they speculated that…” Such a rendition would accurately represent the contents of our paper.

Lines 83-86. Roddy et al. expose here the thrust of their paper and their sampling strategy. However, they refer to ‘species’ and ‘species x group’ without explaining that they sampled individuals from one population of each of only two species. This is mentioned later but should be mentioned here alongside a fair account of Harris and Pannell’s sampling. Such would allow the reader to evaluate criticism of Harris and Pannell’s paper within the context of their sampling scheme: they sampled 20 males and 20 females from 49 species of Leucadendron, whereas Roddy et al.’s paper is based on sampling a maximum of about 8 individuals from each of only two species.

Lines 133 and 134: Roddy et al state: “Harris and Pannell (2010) argue that there is a tradeoff between ramification and hydraulic efficiency”. Again, this is too strong a claim. Our only explicit statement in the Discussion about a trade-off was as follows: “We predicted that if sexual dimorphism has evolved in response to differential resource trade-offs between males and females, then sexual dimorphism should be more marked in areas of greater resource limitation, particularly water”. Note the conditional in our reference to potential trade-offs. Harris and Pannell never referred to a ‘hard trade-off’, which is a particular focus of Roddy et al.

Lines 152-154: Roddy et al. state: “We conclude that sexual dimorphism in plant architecture requires alternate explanations than differences in hydraulic efficiency.” The implication here is that they have arrived at a novel conclusion, and that Harris and Pannell only considered hydraulic efficiency. However, as pointed out above, this is just not true. We, and others before us (including one of the authors of the current manuscript), had already noted that other factors are likely important in shaping patterns of sexual dimorphism in Leucadendron. Critically, the patterns documented by Harris and Pannell requires an explanation. We speculated on the possible importance of hydraulic relations, and mentioned that we might be wrong. But it is simply not logical to reject a hypothesis based on a pattern found among 49 species on the basis of a very small sample on individuals from only two species. The absence of evidence is not evidence for absence, not least when sample sizes so much smaller than in the original study, which of course had much more power.

Methods, Plant material
Line 157: Please state how many individuals of each sex and species were sampled; the range would be most useful. (Note to editor: nowhere is this information supplied in the manuscript, apart from what one may glean from the figures. Roddy kindly sent me his dataset, on which my comments about sample size above and below are based.)

Line 161: “Plants were collected and measurements were made…”. Please give detailed information about how the plants were chosen, and the lengths of the stems that were cut and subsequently measured. Roddy kindly sent me, in an email, the sampling methods used: “We randomly chose individuals in the population, trying not to sample ones that might be size outliers and choosing ones that looked 'average' and healthy.” This sampling protocol is problematic. First, the description is self-contradictory; “trying not to sample ones that might size outliers…” etc. is not random. It is clear that this sampling protocol could NOT have allowed a truly representative sample of the individuals in the populations sampled. In contrast, Harris and Pannell selected individuals along taped transects through their sampled populations, and they did not avoid individuals that might have not ‘looked ‘average’’. In any case, it is important that the sampling approach is reported accurately as performed, so that readers can judge results in the context of the limits imposed by potentially unrepresentative sampling.

Quantifying ramification and other traits
Roddy et al. compared the Harris and Pannell method (‘HP’ in the manuscript) for measuring sexual dimorphism in branching architecture with an alternative method based on the numbers of branch tips (‘BTSA’). They conclude that the HP method is poor. This comparison is not altogether uninteresting. However, unfortunately Roddy et al. did not apply our method optimally. First, although not described in the manuscript (although it should be reported), Roddy explained in email correspondence that branches were measured after being cut, and that only the top portion of the plants as measured. The potential strength of the HP methods is that it considers the pattern of branching of an individual from its apex right down to the first internode near the ground. An apical sample from that full length will give an index of the branching architecture captured by the HP methods, but such an index will be more variable than one based on the full stem length (which allows a regression of points only at the extreme end of the gradient), and so the power to detect pattern will have been even more limited than simply due to small sample sizes. The data kindly sent to me by Roddy also reveals that the branches measured were very short, and thus either that the plants were very young, or, more likely, that only a small part of the upper stem was measured. This would not have been ideal for the HP metric. Moreover, and worryingly, there does not seem to have been any consistent point along the stem at which branches were cut (and subsequently measured); branches differ in the dataset apparently haphazardly, both in their absolute length and in the number of nodes. Application of our approach might have yielded quite different results if it had been applied consistently and according to the same methods we had used. This is an important weakness of the current manuscript, given that its prime focus is a comparison of their metric with ours.

More importantly in the current context, one of the two species assessed by the authors was Leucadendron rubrum, which has a branching pattern that is actually very difficult to assess, especially towards the apex where males produced many tiny branches that do not later contribute to the gross branching architecture with growth in subsequent years. Thus, for L. rubrum, the HP method is appropriate only inasmuch as it focuses on the internal internodes of the plant. A subsample of the stem length might be acceptable if it ignored the tips, not if it focused on the tips. In email correspondence with Roddy, he confirmed the difficulty posed by L. rubrum: “in rubrum males, identifying branches that occur at nodes I found pretty difficult–and even identifying nodes was difficult. There were so many branches on those shoots of all different sizes, even near 'nodes' and nodes in rubrum males are nowhere near as distinct as they are in daphnoides [the other species sampled].” The authors should acknowledge all these concerns and difficulties with their data in their manuscript, so that the reader can form a fair view of how the methods were applied, why there might have been certain differences between Roddy et al. and Harris and Pannell, and, not least, whether the HP method, as applied by Roddy et al., was even appropriate for the material collected, especially in the case of L. rubrum.

Lines 209-211: Roddy et al. state, concerning Harris and Pannell’s widely used metric of sexual dimorphism: “This metric of quantifying the degree of sexual dimorphism is controversial, and we report a more accurate metric of dimorphism”. First, if the method is controversial, then it is important to cite literature in which the controversy is evident, and, even more usefully, to explain its basis. Second, it does not follow logically that a method that may be controversial is less ‘accurate’ than another method. If the authors want to claim that their methods is more accurate, they need to explain how it is more accurate. I think, fundamentally, this is impossible, because judgement of accuracy requires knowledge of a deviation from some true value that is being estimated, yet there is no God-given measure of dimorphism for any species; all we have are metrics that we are free to construct. In any case, the authors need to justify their claims here by referring to the literature and arguing for why their method is better. Finally, given that Roddy et al. aimed to evaluate the methods used of Harris and Pannell, it would seem more logical to use the metric that Harris and Pannell actually used.

Lines 225-231: At the end of this Results paragraph, Roddy et al. claim that the HP method “erroneously estimated L. rubrum females to be more highly ramified than males”. This statement reveals a biased and somewhat circular perspective. ‘Erroneous’ with respect to what? Surely one should approach a dataset with greater indifference? Males and females of L. rubrum certainly do look very dimorphic, and indeed the species has been claimed to be one of the most dimorphic plants known; the sexes differ very much in terms of leaf size, and in the branching pattern near the tips (as revealed by the BTSA metric in the current manuscript). But that does not mean that internal branching architecture must also be sexually dimorphic, any more than root architecture should be, for instance. When studying the 49 species of Leucadendron, we were somewhat surprised that the branching architecture of L. rubrum, based on the HP metric, was not more dimorphic – but that is indeed the nature of the variation we found. It cannot be claimed that the data are erroneous simply because they do not conform to a prejudiced view based on certain particularly apparent aspects of physiognomy. This sentence should be deleted.

Line 232: Roddy et al. state: “Further support for BTSA as a better metric of ramification…” Again, I think it is fairer and more objective to simply point out that the two methods are different; they measure different things. Consideration of leaf size as a potentially dimorphic trait might make this issue clear. For instance, we might choose to compare males and females in terms of leaf length or leaf width, and could find that leaf width is more dimorphic than leaf length. Both dimensions would provide acceptable and informative components of potential leaf size dimorphism (or its absence), but neither would be intrinsically ‘better’ than the other. The authors should resist inappropriate value statements such as this.

Discussion

First paragraph: Roddy et al. report that they “found no consistent support for hydraulic differences between the sexes”, claiming on this basis that “Our results, therefore, question the relationship between sexual dimorphism in branch ramification and serotiny reported by Harris and Pannell (2010).” This is a non-sequitur. It is indeed interesting that Roddy et al. find no consistent hydraulic differences between males and females, but this has no implications whatsoever for Harris and Pannell’s empirical discovery of a “relationship between sexual dimorphism in branch ramification and serotiny”. That result was very clear, based on phylogenetically controlled comparative analysis of 49 species of Leucadendron. No study at all based a subset of two of those 49 species could logically question the original relationship found”. This paragraph should be largely rewritten. In particular, it might be emphasised that the results of Roddy et al. are potentially relevant only to one speculation made in the Discussion of Harris and Pannell’s paper, not to their key result.

The first paragraph of the Discussion of Roddy et al. ends as follows: “Their results depend, first, on accurately quantifying branch ramification and, second, on hydraulic efficiency varying as a function of shoot size and ramification.” First, it is not clear to me what it can mean for results “to depend on accurately quantifying branch ramification”. Of course, an inaccurate (and especially a biased) measure of any variable could yield spurious patterns. But I do not think Roddy et al. are claiming that Harris and Pannell’s measure was inaccurate or biased in that way. Certainly, Harris and Pannell applied precisely the same approach to the measurement of 49 x 40 = 1960 individuals of Leucadendron that had been chosen along transects with no account given to any perception of how ‘average’ they were. It is not clear to me how this approach could be ‘inaccurate’. I would encourage the authors to revise the way they refer to the Harris and Pannell results in this sense.

Line 277: Roddy et al. state: “Accurately characterizing ramification in ways that reflect obvious differences between sexes and species […] is fundamental…”. This paragraph again reveals a worrying prejudice or bias in the authors approach to their data (and those of Harris and Pannell). What could be the point in choosing a method that simply reproduced ‘obvious differences’? More pointedly again, how should such an approach reflect ‘accuracy’? The authors should change their perspective on this point, or at least explain the rationale that might link ‘accuracy’ with ‘obvious differences’, i.e., in a way that does not imply a prejudiced perspective to data which seeks assumed patterns and in which metrics that do not reveal them are judged to be ‘inaccurate’. The ensuing statement “The method used by Harris and Pannell (2010) for quantifying shoot ramification did not accurately describe obvious, qualitative differences between sexes and species" seems to imply that Harris and Pannell erred because their dataset did not reveal the patterns that are so obvious to Roddy et al. But see my analogy with leaf dimensions above; a pattern revealed by one metric, or indeed by subjective assessment of what is obvious, does not reject a different pattern revealed by a different metric. At the very least, the authors should be more critical in comparing metrics that measure different things.

Line 296: Roddy et al. claim that the HP method for measuring ramification "predicted greater dimorphism in ramification for the monomorphic L. daphnoides than for the obviously dimorphic L. rubrum". Again, this is prejudiced in terms of what might appear obvious. There are so many examples in science where what seemed obvious to everyone, even to the greatest minds, turned out not to be correct. We should at least attempt to avoid such pitfalls by not explicitly appealing to the validity of what might appear to be 'obvious'.

Line 335-337: Roddy et al. claim that "there is more variation in sexual dimorphism among weakly serotinous Leucadendron species (those that hold their cones for one year or less) than there is among all other species that maintain their cones for longer than one year", suggesting that this would further suggest "that there is no hard tradeoff (sensu Grubb 2016) between serotiny and branch ramification (Fig. 3 in Harris and Pannell 2010)". I do not follow the argument here. First, it is not adequate to refer to 'more variation in sexual dimorphism among' certain species without being explicit about what character is being measured. Species can be very dimorphic for one trait and not at all for others. The authors should be more restrictive in their statements, because, at least in this case, a general statement is rather meaningless. Second, the implication, in citing Harris and Pannell and their Figure 3, that we claimed a 'hard tradeoff' is puzzling. Readers who take the trouble to look at that figure will see that there is indeed quite a lot of variation in sexual dimorphism in ramification among non-serotinous species as measured by the HP metric, as acknowledged by Roddy et al. But they will also see that, using the HP metric, there is also substantial, and perhaps more, variation among species that differ in serotiny. The relationship between the positive correlation found by Harris and Pannell between ramification and serotiny and the 'hard tradeoff' that Harris and Pannell are incorrectly claimed to assert needs to be clarified.

Lines 349-351: Roddy et al. state: "if the hydraulic architecture of males were less efficient, then males may incur costs associated with reproduction equal to or higher than those of females, contrary to most theory and data regarding the costs of reproduction (Bond & Maze, 1999)." This sentence is somewhat bewildering to me. Which 'most theory' do the authors have in mind? At the very least, this theory should be cited and evaluated against the theory that does not belong to the 'most'. Is some of this theory correct and some wrong? If so, in which way? Or is it that the divergent theory rests on different assumptions? The reader needs some guidance, please. Further, to what does 'most data' refer? This is a bold statement that requires more than one reference. If there are data that run to the contrary (which the authors' statement applies, because 'most' is not all), then the logic of their inference here fails. The fact is that males do sometimes, and perhaps often, incur greater costs of reproduction, as anyone who is familiar with the animal literature will know. Ironically, the only paper cited by the authors to justify their bold (and I believe erroneous) claim here (i.e., Bond and Maze 1999) actually finds greater costs of reproduction for males. Perhaps I have missed or misunderstood something. If so, the authors should be clearer.

Concusions

The concluding paragraph of Roddy et al. makes some important statements about the multiple factors that likely shape sexual dimorphism in Leucadendron. However, I fear that the naïve reader will be led to conclude from this paragraph, once again, that Harris and Pannell suggested only one factor to explain the patternsx their study revealed. But as noted above, Harris and Pannell explicitly cited several factors, not just one. They also explicitly entertained the possibility that their speculation about a possible link between serotiny, water relations and sexual dimorphism might not in the end be sustainable.

Lines 374-376: Roddy et al. end their manuscript with this statement: “until all the possible factors influencing shoot architecture are more comprehensively studied, there may be little clarification to understanding how and why Leucadendron exhibit such extreme and variable sexual dimorphism.” With all due respect, this seems a little rich a conclusion for paper based on the analysis of data from a few ‘average’ individuals sampled from a single population of each of two species of Leucadendron, yet set up to discredit a study of morphological variation among many more individuals in each of 49 species that revealed a surprising and novel pattern in need of explanation. I would welcome new data and new ideas that might help to explain the pattern. But I am disappointed that the authors of the current manuscript attempt to discredit our work by misrepresenting our honest speculation as narrow and single-minded. Even a cursory reading of the Discussion of Harris and Pannell will reveal that it otherwise.