# Doubtful pathways to cold tolerance in plants

Zanne *et al.*[1,2] addressed an important evolutionary question: how did flowering plants repeatedly enter cold climates? Herbaceous growth, deciduous leaves, and narrow water-conducting cells are adaptations to freezing. Using phylogenetic analyses, they concluded that herbs and narrow conduits evolved first in the tropics ("trait first"), facilitating movement into freezing areas, but that deciduous leaves evolved in response to freezing temperatures ("climate first"). Unfortunately, even after correcting for an error that we uncovered[3], the "striking findings" of Zanne *et al.*[1] seem inconclusive; here we highlight methodological issues of more general interest and question the value of their approach. There is a Reply to this Brief Communication Arising by Zanne, A. E. *et al. Nature* **521**, http://dx.doi.org/10.1038/nature14394 (2015).

Zanne *et al.*[1] chose methods that required transforming quantitative variables into binary characters; not surprisingly, we found that their results are highly sensitive to how characters are scored. This is not inherently problematic, but the delineations must be well justified. While we have concerns with each of their thresholds, the climate character underlying their analyses merits special scrutiny. Of the species Zanne *et al.*[1] studied, 50% were represented by collections from both freezing and non-freezing areas; these were scored as "freezing-exposed" if 2.5% of the collections experienced a minimum

temperature of 0 °C. This cut-off used the tail end of species distributions to delineate character states, where we expect considerable error in the data[4,5], especially for the many poorly collected species in their sample. Using more stringent data cleansing and/or alternative thresholds for "freezing-exposed", we obtained a wide range of results (Fig. 1a). For instance, when we required half of the collection sites to experience freezing, the leaf phenology result shifted from 36.7% to 72.5% trait first. Depending on how climate data were handled, results for plant habit varied from 25.3% to 95.5% trait first (see https://github.com/ejedwards/reanalysis_zanne2014) and, contrary to Zanne *et al.*[1], we sometimes found that growth form was twice as evolutionarily labile as climate occupancy.

But our concerns run deeper. Their evolutionary trajectories were inferred using a newly developed method whose behaviour is unexplored. In simulations we discovered that their method strongly infers a preferred trajectory even when none is present (see https://github.com/ejedwards/reanalysis_zanne2014). When the simulated data contained an equal number of climate-first and trait-first transitions, their method inferred a strong climate-first or trait-first trajectory 77% of the time (Fig. 1b). Thus, the preferred trajectories of Zanne *et al.*[1] could have nothing to do with what actually happened during angiosperm evolution, and no attempt was made to connect their
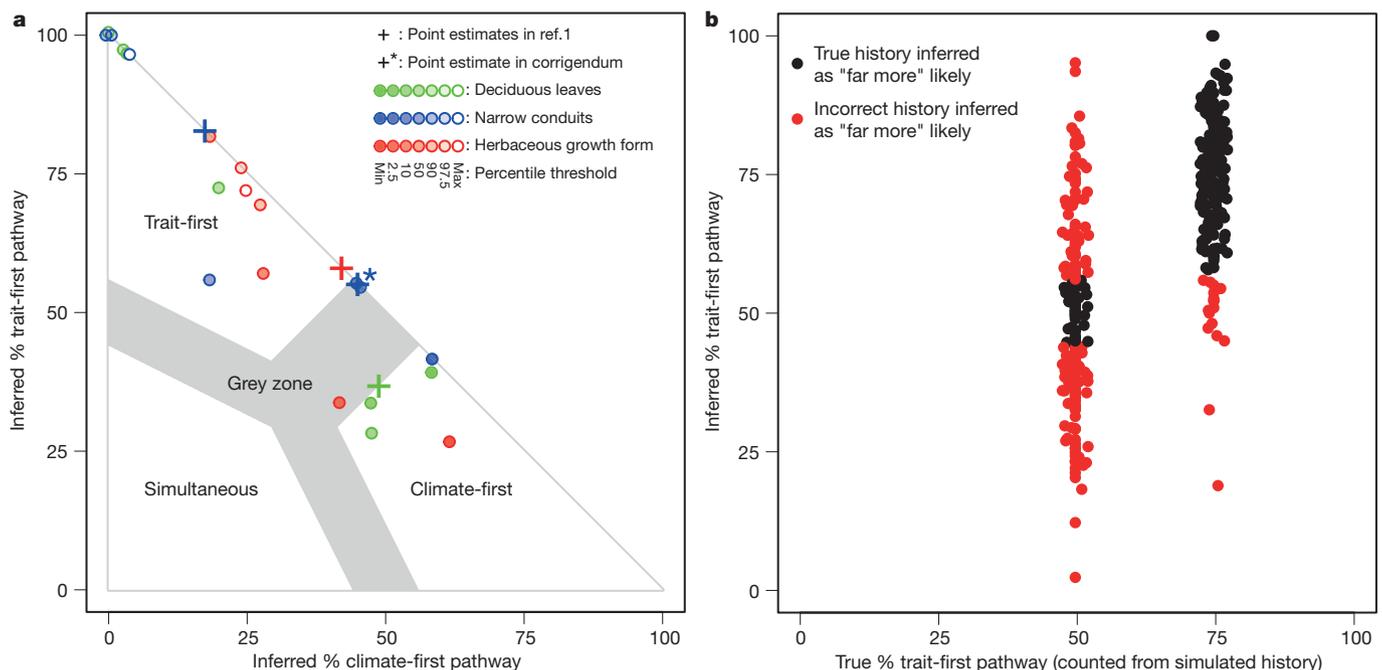


**Figure 1 | Unreported uncertainty and potential error.** Based on the analyses by Zanne *et al.*[1]. **a**, Sensitivity of the Zanne *et al.*[1] results to alternative treatments of the climate data. For each of the three traits, the Zanne *et al.*[1] result is marked by a cross (48.7% "climate first" for deciduousness; 82.7% and 58.0% "trait first" for conduit size and growth form, respectively). Our re-analysis of conduit size using the correct diameter variable shifts their inference to 53.5% trait first (marked with an asterisk), moving their only strong result into a region of questionable significance (the "grey zone"), along with their other two pathways. For each trait we obtained a wide range of outcomes, including apparently decisive support for climate first or trait first, by simple modifications of the climate variable (see Methods). This figure includes only our implementation of different freezing thresholds; for the effect of alternative

data cleansing see https://github.com/ejedwards/reanalysis_zanne2014. **b**, Error rates using the Zanne *et al.*[1] transition-rates method. We simulated character evolution with a strongly biased pathway (3 times more "trait-first" transitions) and with no preferred pathway (equal number of "trait-first" and "climate-first" transitions) to examine the behaviour of their method. When there was a strong underlying trajectory in the data, their method could usually detect it. However, when there was no dominant trajectory, their method performed poorly, incorrectly inferring a strongly preferred pathway 77% of the time. Zanne *et al.*[1] described deciduous leaves as being "far more likely" to have evolved climate first (49% vs 37%); on this basis we considered one pathway "far more likely" than another if the difference was 12% or more.

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trajectories to inferred character changes in the phylogeny. Consequently, it is unclear, even roughly, how many tropical-to-temperate transitions were sampled, or how their trajectories relate to the directionality of change. In the case of their erroneously binned conduits, for example, the few taxa they scored as having the supposed ancestral condition were instead recently derived within the tree.

Finally, we disagree with Zanne et al.[3]'s claim that their results are "qualitatively the same" after correcting for their error. 54% and 83% seem like very different answers, and all of their preferred pathways now hover around a grey zone where their probability is hardly greater than the alternatives (Fig. 1a). In the end, we struggle even to understand the meaning of a number like 54%. It should not be taken to mean that 54% of transitions were trait first when, as we have demonstrated, their method cannot accurately infer the true evolutionary history. Nor should we interpret their result as if every species had a 54% chance of a trait-first transition, when their own sub-analyses of growth form showed that these probabilities vary widely by clade. We urge greater caution in conducting and interpreting phylogenetic analyses at this scale, and predict that robust generalizations about the history of life will emerge from analyses of multiple, carefully developed case studies that incorporate more of the relevant variables[6].

## Methods

We employed seven different thresholds to define a species as "freezing exposed", using various percentiles of localities experiencing 0 °C. We excluded duplicate records, enforced minimum sample sizes ($n = 3$, $n = 10$), performed alternative data grooming procedures, and re-ran the original analyses across all data sets. We also simulated character histories with differing degrees of bias towards particular pathways. We scored the relative frequency of the trait-first pathway from each simulation, and compared it to the trait-first probability inferred using their method. Annotated scripts and analyses are publicly archived in https://github.com/ejedwards/reanalysis_zanne2014.

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# Zanne *et al.* reply

Our goal was to understand which traits facilitated angiosperm shifts into freezing climates. Building on previous work[1–5], we showed strong support for evolutionary shifts in herbaceous habit, deciduous leaf phenology and small water-conducting conduits with the transition to exposure to freezing for the first time at this scale. We then decoupled the order of these shifts (traits-first versus climate-first pathways) based on a new summary of a long-standing method[6] with no a priori expectations. Because current data sets are small compared to estimates of angiosperm diversity, our pathways analyses are preliminary. By establishing testable hypotheses and making our considerable resources public, future studies can build upon our questions. We found their suggestion in ref. 7 that we looked for reifying patterns in nature surprising. In the accompanying Comment[8], Edwards et al.[8] reanalysed data from Zanne et al.[9], including removing data points and using new thresholds (below). After correcting an error in conduit size threshold[10], we still found that "trait first" was the most likely pathway, albeit with less strength. Otherwise, we stand by the validity of our approaches.

Ancestral state estimates are notoriously unreliable[11]. Rather than using estimates at hundreds or thousands of nodes, we used the presumably more reliable, inferred 8–12 transition rates to examine likely pathways. If the rate of going from state A to B is three times the rate of going from A to C for 100 species starting in state A, we expect 75 to go to B first and 25 to go to C first. This expectation follows directly from a summary of the rates. Calculations are more complex for four states, but result in the same information: converting rates to expected paths.

Edwards et al.[8] performed simulations that showed this summary was not biased, but that known paths may deviate, at times substantially, from this expectation, especially if rates are similar.

We agree that various thresholds are potentially suitable[8]. We disagree that radically changing thresholds should reveal the same result; Edwards et al. varied cut-offs from requiring 0% to 100% of a species range experiencing freezing (see figure 1 in ref. 8) for that species to be freezing exposed. A priori, we targeted >2.5% of a species range. Edwards et al.[8] targeted >50% of a species range. Both are valid and selection should be guided by the biology of the system. Under our definition, if a species experienced freezing somewhere, it had the potential to handle freezing (a species-specific trait, not unlike our leaf and stem traits). Owing to the limitations of GBIF coverage, we believe it was better to define species as experiencing freezing (with >2.5% allowing for outliers) rather than to expect a set amount of a species range to be in freezing.

We agree that narrowly defined case studies provide detailed insights into a given lineage[8]. Equally important, large-scale analyses afford synthesis, examining broad evolutionary hypotheses missed by narrow studies. These approaches are certainly complementary, each with strengths and weaknesses, and it is critical that studies continue to be conducted across multiple scales.

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