

Reply

Is Wing Recurrence *really* impossible?: a reply to Trueman *et al.*

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By using multiple molecular markers and employing several methods of tree reconstruction and character optimization, we demonstrated that the ancestral phasmid is reconstructed unambiguously as wingless, with wings being reacquired later in phasmid evolution (Whiting *et al.*, 2003). We presented this as a compelling example of recurrence in which a complex character, once lost to evolution, is regained subsequently in a descendant lineage (West-Eberhard, 2003). Our hypothesis is refutable via additional phylogenetic analyses including a larger selection of taxa, additional molecular markers, morphological data, or by examining patterns of development of wing expression in phasmids. We are currently performing research in each of these areas to add greater precision to this hypothesis.

Trueman *et al.* (2004) have not presented a formal test of our hypothesis, nor contributed additional data to refute our findings. Wing recurrence is a hypothesis of character transformation, requiring a phylogenetic topology for interpretation. The 'traditional view' of phasmid wing evolution that these authors embrace was conjured in phylogenetic ignorance, since we presented the first formal analysis of phasmid phylogeny. Clearly the current data support a basal placement of apterous taxa, and multiple researchers who have reanalysed our data, including Trueman *et al.*, have been unable to find a topology which rejects this hypothesis, regardless of analytical methodology. Thus, Trueman *et al.* quibble over methods of character optimization by launching into confused, non-phylogenetic, and mutually contradictory arguments to unravel our hypothesis 'before this extraordinary evolutionary scenario reaches the entomology textbooks'.

When the cost of wing gain is set extremely high (parsimony), or the rate of transformation from wingless to winged is set extremely low (likelihood), any method of character optimization will bias against reconstructing wing recurrence. More generally, values always can be selected to make it impossible to detect character recurrence by forbidding its transformation on a phylogenetic topology (= Dollo's law). Because we believe that phylogenetic topologies should establish patterns for inferring evolutionary processes, the issue becomes how much evidence is required before recurrence is a well-supported hypothesis. Our analyses

and those of Trueman *et al.* agree on one critical point: under both parsimony and likelihood methods of character optimization the ancestral stick insect is supported unambiguously as wingless, with wings gained on multiple occasions.

Trueman *et al.* (2004) premise their argument with the curious statement that 'reconstruction of the phasmid ancestor is not the relevant issue.' We argue it is the only relevant issue. If an ancestral node is reconstructed as wingless, and a descendant node is reconstructed as winged, then there must be a transformation from wingless to winged. Gains and losses are not observations: they are inferences based on hypotheses of character transformations given a topology and method of character optimization. To discard ancestral reconstruction is to discard the very reason why phylogeny is critical for investigating character evolution. Trueman *et al.* fail to follow their own dictum in discussing the relative merits of hypotheses using values obtained directly from character optimization (e.g. four gains and three losses for the wing 're-evolved' hypothesis). Moreover, if all that matters is how well the 'model fits the data' then we can dispense with their parsimony arguments outright, as most systematists would acknowledge that parsimony is not attempting to quantify the fit of data to specific evolutionary models.

Trueman *et al.*'s criticisms can be summarized as three points: (1) the 'probability' of wing loss to gain should be 2.5 under parsimony; (2) the 'probability' of wing loss to gain should be 6 : 1 under parsimony; and (3) the 'probability' of wing loss to gain should be 13 : 1 given a likelihood analysis.

Probability of 2.5 : 1

Trueman *et al.* argue that the ratio of wing loss to gain should be 2.5 : 1 (or 2 : 1 if nodes are 'alternatively resolved' by an *ad hoc* rearrangement of taxa to minimize character transformations). This is based on the supposition that 'Because three losses are common to both scenarios the relevant comparison is between four gains for one hypothesis and ten losses for the other, a ratio of just 2.5 : 1'. However, the three losses are not common to both hypotheses *because they are not the same character loss*. The hypothesis of wing gain places a loss at the base of Phasmida; the alternative hypothesis does not. Their mistake is obvious if a ratio of 2.5 : 1 is entered in MacClade and the ancestor to phasmids remains unambiguously

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wingless. These values make sense only if phylogeny is eliminated from the equation.

Probability of 6 : 1

We reported that if wing gain costs six times that of wing loss, then the ancestral node is reconstructed as winged with multiple, independent losses postulated. More precisely, when cost of gain equals one to four times cost of loss, parsimony reconstructs the basal phasmid unambiguously as wingless. When gain equals five times that of loss, the ancestral condition is ambiguous; when wing gain is equal to six times loss, the ancestral condition is winged. This is the appropriate way to evaluate the relative merits of either hypothesis under parsimony because it evaluates characters in reference to a topology. Trueman *et al.* suggest that this cost ratio (which they equate with probability) is low relative to their intuitive feelings about the 'probability' of gain vs. loss. They fail to specify, however, what cost ratio is acceptable or how this cost ratio is computed from their intuition. That these values appear low under parsimony is not surprising because parsimony is a very conservative method of character optimization (Pagel, 1999). Nonetheless, a 6 : 1 cost ratio is the highest value ever reported in the literature for character recurrence, and is compelling because this ratio correlates with the placement of six apterous lineages in succession at the base of the topology. In fact, in order to find wing recurrence under a cost ratio of 1000 : 1, one must find a topology which for practical purposes is unobservable: 1000 successive interior nodes optimized as wingless, with a winged, more apical node.

Likelihood analysis

When alpha and beta are allowed to freely vary in the program DISCRETE, as advocated by Pagel (1999), our analyses and those of Trueman *et al.* reconstruct an ancestral apterous stick insect with subsequent wing recurrence. Given this likelihood approach, the model of a wingless stick ancestor with wing recurrence is thus a better fit to the data than that of a winged ancestor with multiple losses. Our results and those of Trueman *et al.* disagree on the degree to which beta can vary and still support this conclusion, but this is a non-issue because the parameter beta is an instantaneous rate (and not a probability) and depends only upon branch lengths and phylogenetic distribution. Moreover, we question their reanalysis on the following grounds. (1) Their likelihood tree is different from ours and they did not specify how it was obtained. Our likelihood results were based upon extensive analyses using a SP2 supercomputer executed in parallel. (2) We performed DISCRETE on a set of thirty trees under Bayesian analysis to detect potential biases based on the selection of an individual tree and found similar results; they never address this. (3) Their DISCRETE data matrix inexplicably omits an outgroup to polarize character states and is thus not directly comparable to ours, and (4) we are unable to replicate the values they report given the DISCRETE matrix

that they provided us with. Nonetheless, despite these differences, we are encouraged that they have also found that the wingless ancestor is supported under likelihood analysis.

Conclusions

Recurrence is an under-appreciated but potentially widespread evolutionary phenomenon. Recurrence has been suggested for the re-evolution of eyes in ostracods (Dingle, 2003), ocelli in cave crickets (Desutter-Grandcolas, 1993), wings in water striders (Anderson, 1997), wings in male *Philotrypesis* fig wasps (J. Greeff, pers. comm.), and other complex features in a wide variety of taxa (reviewed in West-Eberhard, 2003). No developmental biologist has ever expressed to us concern that wing developmental pathways could be conserved over long evolutionary time periods in apterous taxa. Only systematists embrace these evolutionary assumptions to argue that recurrence is unlikely. Our study received such widespread attention because it stands as the most compelling case of recurrence to date. The placement of multiple lineages of wingless taxa at the base of the phasmid topology was a pattern which unexpectedly emerged from the phylogenetic reconstruction, no other winged insect order has even a single apterous lineage that is placed basally, and regardless of how you manipulate these data or argue over numbers, one must still account for this unusual pattern. Trueman *et al.* are content with a grand *ad hoc* dismissal of this pattern as widespread reversal and provide no criterion which would ever reject their explanation. Our view of stick insect evolution may change with additional data, but we maintain that the current data and analyses stand as the best supported case for evolutionary recurrence.

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