Comparing Plants and Connecting Traits

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Comparing plants and connecting traits

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SUMMARY

The diversity of plant life histories provides a wealth of raw material for comparative studies on evolution and ecology. The two fundamental questions for any comparative study are: which traits are correlated with one another, and are these correlations the result of common descent or convergent evolution? Phylogeny should therefore be explicitly included in any comparative analysis that is concerned with the *causes* of correlation between traits, even when the principal research question is a purely ecological one. In illustration, the method of phylogenetically independent contrasts (PIC) is used to test two long-standing hypotheses that have not been satisfactorily tested before. In the first example we find that annuals and species of early succession have greater reproductive allocation than perennials and species of later succession. In the second example we show that the apparency hypothesis of chemical defence is supported by a positive correlation between woodiness and the frequency of tannins and by a negative correlation between tannin frequency and alkaloid frequency. Finally, we point out that PIC has a much lower type-I error than cross-species analyses and that this superiority is surprisingly robust to lack of phylogenetic resolution.

1. INTRODUCTION

Plants arguably display a greater range of life history variation than is found in any other kingdom and this diversity provides a wealth of raw material for comparative studies on evolution and ecology. In this paper we will first make some general and methodological points about using the comparative method to seek trait correlations in plants and then describe two new comparative analyses of our own by way of illustration. The closing remarks will consider the relative merits of phylogenetic *vs* non-phylogenetic methods of comparative analysis.

The two fundamental questions for any comparative study are:

1. Which traits are correlated with one another?
2. Are trait correlations the result of common descent or of convergent evolution?

The first of these questions is routinely asked by anyone interested in plant life history, whether they are primarily interested in an ecological or an evolutionary interpretation of the answer, but the second question is asked by plant ecologists much less frequently than it ought to be. Why, many plant ecologists seem to ask, do we need to worry about phylogeny at all if we are not asking an evolutionary question? More subtly, Westoby *et al.* (1995 and this volume) have argued that phylogenetically based comparative methods are conservative in their interpretation of the evidence for adaptation and that correlations based on species as independent data points give the *ecological* independence of species from one another their due weight. Harvey (1996; Harvey *et al.* 1995) has recently

answered this question quite comprehensively, so the answer given here will be as brief as possible.

A typical ecological question is, do the species found in one type of habitat have larger seeds than the species found in another type of habitat? Several studies have addressed this sort of question and, using each species in their samples as an independent data point, they have concluded for example that species living in dry habitats have larger seeds than those in wetter habitats (Baker 1972) and that light-demanding pioneer tree species have smaller seeds than species of mature tropical forest (Foster & Janson 1985). If one did not want to interpret these correlations causally, then there would be no problem with this approach, but of course one *always* wants to explain *why* mean seed size differs between habitats and it is this that produces the problem. Since all species have a common ancestor at some point in their evolutionary history, there will always be some degree of ancestral similarity between the species sharing a habitat, and common ancestry, not adaptation, may be responsible for similar seed size. Species sharing a habitat may also have similar seed sizes as an accidental consequence of an association between seed size and some other trait. To put it in statistical terms, common descent causes traits to be confounded with one another when species are the data points. If two species share similar traits due to common descent, then treating them as independent data points when testing for trait correlations is the equivalent of pseudoreplication in a designed experiment (Rees 1995).

There seems to be a common misconception that the argument just summarized applies only to causal hypotheses that invoke natural selection (e.g. shade

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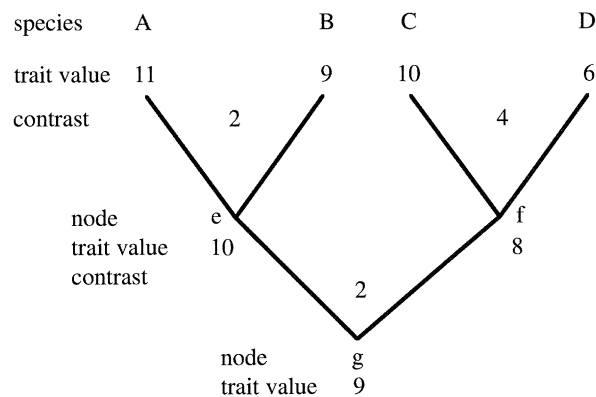*Printed in Great Britain*

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Figure 1. A phylogenetic tree for four species (A–D). Contrasts are calculated as the difference between the trait values of the two species that branch from a common node. Trait values may be assigned to internal nodes of the tree by assuming an evolutionary model of character evolution. Under the Brownian motion model, a node's trait value is the mean of the values for the two species (or nodes) that branch from it. In the example shown all contrasts are positive, but in reality some traits will show negative contrasts on the same tree. Also, in this example it has been assumed that branch lengths are equal though the method can use branch length information if this is available.

selects for large seed size), and that it does not invalidate tests of hypotheses invoking ecological sorting (e.g. only large-seeded species can colonize shaded habitats) as a cause of trait/habitat correlations (Westoby *et al.* 1995). However, if common descent confounds traits, any test for trait correlation that ignores this will be jeopardized, regardless of the causal hypothesis being tested.

The problem, then, is how to deal with the confounding effects of common descent. The best solutions to this problem involve using a phylogeny for the taxa being compared. Take a phylogenetic tree for a group of four species A–D in which we want to test whether the value of two traits are correlated (figure 1). Felsenstein (1985) pointed out that differences between species A and B will be independent of differences between C and D because in each case those differences arose after the common ancestor of all four species lying at the node marked 'g'. We can therefore treat those differences, technically called contrasts, as phylogenetically independent of one another. Furthermore, if we use an evolutionary model to estimate the value of the traits at nodes 'e' and 'f', then the contrast between these nodes is also phylogenetically independent. By working down an entire phylogenetic tree in this way, a phylogeny for *n* species can provide *n*–1 contrasts for hypothesis testing. By testing for correlation (for quantitative traits) or association (for qualitative traits) between trait contrasts instead of between trait values, the effects of common descent can be removed.

If there is no phylogeny available, then one solution to the problem is to confine comparisons to congeneric or confamilial pairs of species because we can usually be pretty confident that these share a more recent common ancestor than species in other genera or other families (the only doubt can be over whether taxonomic classification accurately reflects phylogeny). Salisbury (1942) used this technique on congeners to look for associations between seed size and habitat many years ago and, more recently, Kubitzki & Ziburski (1994) used intra-generic comparisons to show an association between seed dispersal type and

habitat in tropical species for which no phylogeny was available.

2. EXAMPLES
(a) *Reproductive allocation*

Our first example looks at patterns of reproductive allocation (RA) and asks, does the proportion of dry weight devoted to reproductive structures vary with plant life history and does it vary with the successional stage of habitats? These were fashionable questions 20 years ago, but the steam rather went out of the subject when people began to compare results for different species and from different studies and realized that there were a host of methodological questions, such as whether root biomass had been measured or not, that made it difficult to draw general conclusions. We do no more than mention the methodological problems here because they are secondary to our purpose and they have been comprehensively reviewed by Willson (1983). In a quandary, she noted that exceptions to the expected patterns were legion: 'We do not know if these are unusual populations of the species or if these plants are true exceptions to the "rule".'

Suffice it to say, many of the difficulties can be overcome if comparisons are designed to control for the phylogeny of the species and for the authorship of the study. Surprisingly, neither of these controls has been systematically applied in a comparative study of plant reproductive allocation before. Phylogenetic control can ensure that the major differences between taxa, for example in the size of the infructescence and other ancillary reproductive structures, do not overwhelm differences that correlate with life history. By confining comparisons to those possible within the results of individual studies at least some of the methodological differences between authors can also be eliminated.

One of the largest and most frequently cited studies of reproductive allocation was by Abrahamson (1979) who measured RA in 50 herb species collected from old fields and woods in Pennsylvania. He reported both that species of old fields (i.e. early succession) had a

higher RA than did woodland herbs (later in succession) and that old field annuals had a higher RA than old field perennials, but his analysis treated each species as an independent data point. This comparative method is referred to as TIPS (because all the tips of the phylogenetic tree are compared), in contradistinction to PIC (phylogenetically independent contrasts).

We have tested the two patterns reported by Abrahamson using PIC on his data, supplemented by data from other sources. Thirteen of the 50 species in Abrahamson's study belong to the family Asteraceae, though only one of these occurred in woods. The phylogeny of these species (figure 2), based on the molecular phylogeny of Jansen *et al.* (1990), illustrates three contrasts that can be used to test the patterns reported by Abrahamson. Neither the RA contrast between field and woodland species of *Hieracium* ($t_{18} = 0.745$, $P > 0.05$) nor the contrast between the annual *Erigeron annuus* and the mean RA of the two most closely related perennials ($t = 0.94$, $P > 0.05$) is significant. The contrast between the mean RAs of annual *Ambrosia* spp. (8.5%) and perennial *Rudbeckia* spp. (16%) is significant, but in the wrong direction for the hypothesis ($t = 6.39$, $P < 0.001$).

One other contrast between annual and perennial species in the same family (Primulaceae) was available in Abrahamson's dataset: the contrast between the annual *Anagallis arvensis* (RA $= 38 \pm 12.4$) and the perennial *Lysimachia ciliata* (RA $= 3.0 \pm 1.5$) was highly significant ($t_{18} = 8.86$, $P < 0.001$) in the expected direction. We also looked for annual/perennial contrasts in other studies that could be used to test this pattern and found 10 further pairs distributed among eight families. Analysed as a group (including the Abrahamson contrasts), these data strongly support the hypothesis that annuals have a higher RA than perennials (figure 3*a*) (Wilcoxon matched pairs test, $n = 13$, $t = 8$, $P < 0.009$). The data of Wilson & Thompson (1989) on the RA of annual and perennial British grasses were not included in the analysis, but also strongly support this result.

A total of five confamilial contrasts were available from Abrahamson's data to test his successional pattern. Two of these contrasts were significant in the predicted direction and three were not, but overall the null hypothesis of no association between habitat and RA could not be rejected (Wilcoxon matched pairs test, $n = 5$, $t = 5$, $P = 0.50$).

Hancock & Pritts (1987) reviewed the literature relevant to Abrahamson's two hypotheses and tabulated the RA of 30 perennial species belonging to the Asteraceae, 11 of which are found in early successional habitats and the remainder in later succession. Confining comparisons to those within individual studies as before, this dataset includes seven phylogenetically independent contrasts between early and late successional species (figure 3*b*). If phylogeny and authorship are ignored, there is no significant difference in RA between the two groups, but when data points are matched, the difference is significant in the direction predicted by Abrahamson's hypothesis (Wilcoxon matched pairs test $n = 7$, $t = 2$, $P = 0.043$).

The phylogenetically controlled tests of Abrahamson's two hypotheses demonstrate how independent contrasts can be used to clarify what were two very messy relationships based on pseudo-replicated samples (i.e. lacking in phylogenetic control) and with many apparent exceptions. Exceptions remain of course, but even these may prove to be more informative now that the patterns have been soundly established.

(*b*) *Secondary chemistry and life form*

Our second example is altogether a much more ambitious one. Rapid progress is now being made in constructing a molecular phylogeny for the families of flowering plants and this provides the exciting opportunity to look at large-scale life history patterns across the angiosperms as a whole. In 1993 Chase and 41 coauthors published phylogenetic hypotheses for 265 families of angiosperms based on DNA sequences of the *rbc*L gene in nearly 500 plant species (Chase *et al.* 1993). Though subsequent work has led to a revision of some of the phylogenetic relationships originally proposed by Chase *et al.* (Rice *et al.* 1995), it still forms an invaluable basis for exploring trait variation at the family level and above.

We have used the independent contrasts method and the Chase phylogeny to test the hypothesis advanced by Feeny (1976) 20 years ago that the types of defensive secondary compounds deployed by plants differ according to plants' 'apparency' to herbivores. Feeny (1976) suggested that plants that are more 'apparent' to herbivores should invest in relatively high tissue concentrations of digestibility-reducing compounds such as tannins, while plant species that are less apparent should invest in more toxic compounds such as alkaloids that are poisonous at relatively low concentration. Although this theory is in many ecological textbooks and has been cited over 635 times in the primary literature, there has hitherto been no comprehensive test of the hypothesis. Our test utilizes data on the phylogeny and secondary chemistry of over 165 angiosperm families.

An objection that has been raised against the apparency hypothesis is that it is difficult to know what particular features of a plant make it 'apparent' from a herbivore's point of view. For the purposes of the present test we follow Feeny in assuming that woody plants will be more apparent than herbs because of the gross difference in size between the two life forms. If this assumption is incorrect, it is unlikely to bias the test in favour of a positive result. Another objection raised is that the association between the tree life form and the presence of tannins, which Feeny noted, could be the product of common descent (Mole 1993). Life form and most kinds of secondary compounds including the tannins and alkaloids are phylogenetically conservative in their distribution, so comparative tests that seek correlations among these variables must establish the phylogenetic independence of any relationship that is claimed.

We used PIC as implemented in the CAIC computer package (Purvis & Rambaut 1995) to analyse our dataset. It is important that the phylogeny used in any
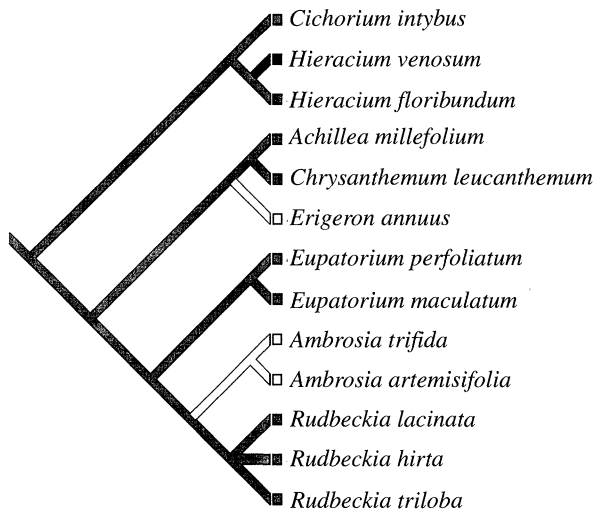
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Cichorium intybus
Hieracium venosum
Hieracium floribundum
Achillea millefolium
Chrysanthemum leucanthemum
Erigeron annuus
Eupatorium perfoliatum
Eupatorium maculatum
Ambrosia trifida
Ambrosia artemisifolia
Rudbeckia lacinata
Rudbeckia hirta
Rudbeckia triloba

Figure 2. Phylogeny of Asteraceae in the Abrahamson (1979) dataset. Annuals shown in white, old field perennials in grey and the single woodland perennial in black. Two annual/perennial contrasts are present, and one field/woodland habitat contrast. The tree was drawn by combining the phylogenetic relationships among tribes given by Jansen *et al.* (1990) with the tribal membership of species given by Mabberly (1987).

such analysis has been derived completely independently of the traits (life form, tannins, alkaloids) under investigation so the Chase *et al.* molecular phylogeny was ideal for this purpose. As generally recommended for comparative analyses (Eggleton & Vane-Wright 1994), the published example of one of the most parsimonious trees (from Search II) was used in

preference to the consensus tree. Fifteen polyphyletic families were omitted from the analysis. CAIC permits branch lengths to be treated in a variety of ways; we chose the option that sets them all equal because even though Chase *et al.* (1993) give branch lengths, it is known that the rate of evolution in the *rbc*L gene varies considerably between different angiosperm lineages (e.g. Bousquet *et al.* 1992 *a*; Frascaria *et al.* 1993).

All data were analysed at the family level or above. We used two compilations of the taxonomic distribution of plant secondary compounds as sources of chemical data: Mole's (1993) tabulation of the proportion of tested species that contained foliar tannins in 224 families; and Levin's (1976) similar tabulation of the proportion of tested species containing alkaloids in 110 families. The tannin data were based on 2227 species and the alkaloid data on 11 299 species. Tropical as well as temperate families were well represented in both compilations. It has been claimed that alkaloids are more common in tropical families than in temperate ones (Levin 1976), but a test of this hypothesis using PIC on Levin's data did not support this pattern. Family synonymies were checked in Mabberly (1987), which was also the source of life form data for each family. Families were scored as 'herbs present' or 'herbs absent', with the latter category including cases where Mabberly described herbs as 'few' or 'rare'.

In the three tests of the apparency hypothesis we asked:

1. Is the presence of the herbaceous growth form in a family negatively associated with the proportion of species in the family possessing foliar tannins? The
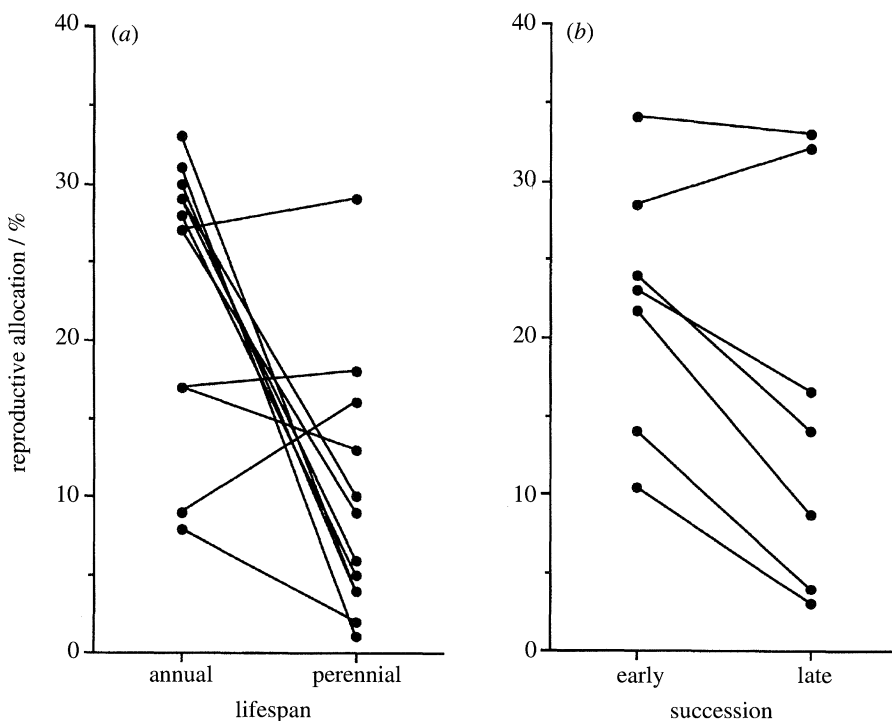

Figure 3. (*a*) RA contrasts for 13 annual/perennial pairs of species (some points overlap). To control for methodological differences between studies, the contrasts shown are confined to species where the same authors had measured RA in both the annual and the perennial. (*b*) RA contrasts between seven pairs of early and late successional perennial species in the Asteraceae. Data are from various sources, listed in Appendix 1 of Hancock & Pritts (1987) and include one contrast from Abrahamson (1979).
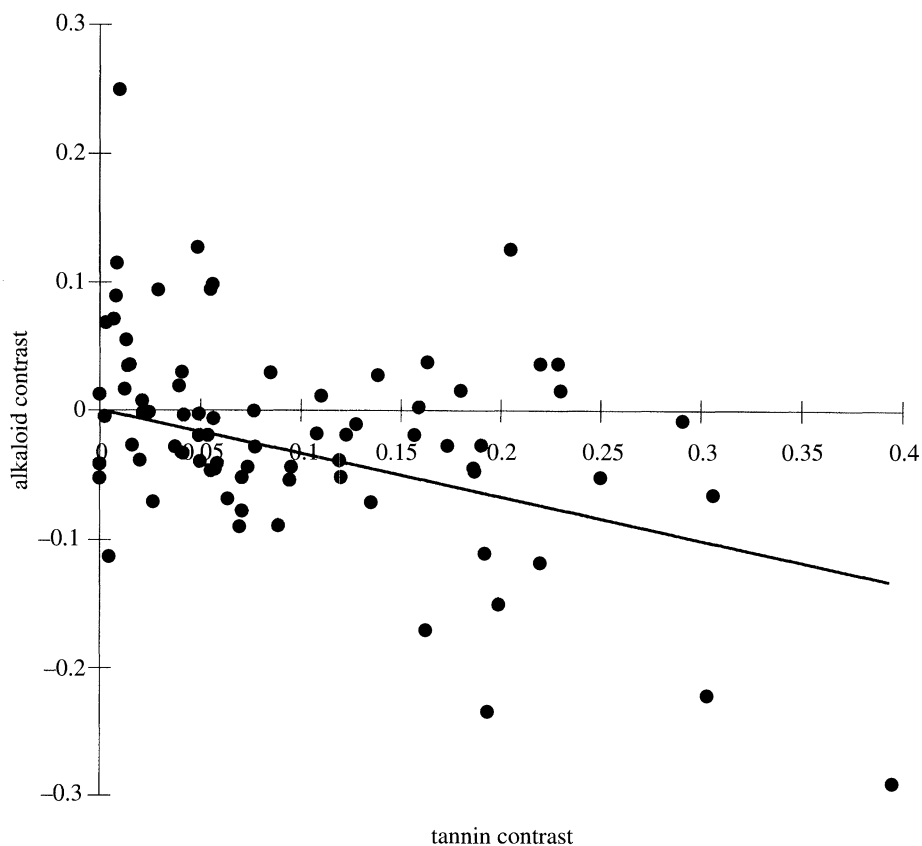
Figure 4. Relationship between alkaloid and tannin contrasts. Regression through the origin: $F_{1,77} = -21.22$, $P < 0.0001$.

answer was 'yes' ($n = 36$, $t = -2.836$, $P = 0.008$, 2-tailed test). It is worth noting that one of the contrasts that went in the 'wrong' direction in this test because tannins were at low frequency in the woody taxa (Morningaceae + Caricaceae (woody) *vs* Brassicaceae and other herb families) fell within the clade of families that contain mustard oil glucosides (Rodman *et al.* 1993). Mustard oils were the toxins Feeny (1976) originally had in mind as defensive alternatives to tannins.

2. Is the presence of the herbaceous growth form in a family positively associated with the proportion of species in the family possessing foliar alkaloids? A positive association was found, but this was not significant ($n = 21$, $t = 1.566$, $P = 0.133$, 2-tailed test). This result was heavily dependent on one contrast (Sterculiaceae *vs* Malvaceae) in the 'wrong' direction for the hypothesis. On average 24% of species in non-herb families contained alkaloids compared with 41% in herb families, but 40% of species tested in the Sterculiaceae (no herbs) and only 16% of species tested in the Malvaceae contained alkaloids. Removing this contrast gives $P = 0.053$ for a 2-tailed test.

3. Is the proportion of species in a family possessing foliar tannins negatively correlated with the proportion possessing alkaloids? The answer was 'yes' (figure 4).

Sample sizes in these tests were smaller than the number of data points for individual variables because in tests 1 and 2, involving the categorical variable

herbs present/absent, only contrasting families or branches were compared and in test 3 sample size was determined by the intersection of the alkaloid and tannin datasets.

Tests 1 and 3 strongly support the apparency hypothesis, test 2 offers weak (or no) support. Feeny's original observation 20 years ago that foliar tannins are associated with the tree life form and are under-represented among herbs is robust and appears to be a genuine evolutionary pattern, not an artefact of common descent. The comparison of tannin distribution with alkaloid distribution (figure 4) strongly supported Feeny's suggestion that these two different kinds of chemical defence are negatively associated. Although these results support Feeny's hypothesis, they do not refute other theories that make similar predictions (e.g. Coley *et al.* 1985).

A recent review of the theory and data on chemical defences concluded that apparency theory and many other hypotheses have all failed to provide a general explanation of why plants deploy particular kinds of chemical defence (Berenbaum 1995). Our test of the apparency hypothesis draws upon the widest possible sample of plants distributed right across the angiosperms and its positive result is therefore cause for optimism that a general theory of plant chemical defences is indeed attainable. Broad, comparative phylogenetic tests of other theories are now called for.

3. DISCUSSION

Phylogenetically independent contrasts has become the method of choice in comparative studies and the examples given demonstrate its utility in answering questions about life history evolution in plants. However, there is resistance to the adoption of PIC among some plant ecologists and the problems of applying phylogenetically correct methods do deserve serious attention. Modern comparative methods demand phylogenetic information that is at present still lacking below the family level for most plants. However, we should not let this blind us to the real problems of using TIPS. Computer simulation studies have demonstrated that TIPS (16%) has a much higher Type I error rate (rejecting a true null hypothesis) than PIC (5%) (Martins & Garland 1991). When a phylogeny is fully resolved, the Type II error rate (accepting a false null hypothesis) is also higher for TIPS than for PIC and PIC therefore has the greater statistical power (defined as 1-Type II error rate). Purvis *et al* (1994) applied PIC to simulated phylogenies varying only in the number of polytomies (unresolved relationships among branches). They found that the Type I error rate of PIC was quite robust to poor phylogenetic resolution, though the Type II error rate (and therefore statistical power) was badly affected.

Harvey & Pagel (1991) and others have suggested that a pragmatic solution to the absence of a phylogeny is to use taxonomic relationships as a surrogate, as a number of comparative plant studies have done (Kelly & Purvis 1993; Kelly 1995; Kelly & Beerling 1995; Kelly & Woodward 1995). Miles & Dunham (1993) caution that taxonomic trees may not be congruent with phylogenetic ones and should not be treated as surrogates for them, however their argument is weakened by the growing number of instances (although with notable exceptions) where molecular phylogenies are found to agree in large part with standard taxonomic treatments (e.g. Bousquet *et al.* 1992*b*; Bremer & Struwe 1992; Patterson *et al.* 1993; Bruneau *et al.* 1995).

More important than the congruence of taxonomy with phylogeny is the general point that phylogenies as well as taxonomic surrogates for them are only hypotheses and that many (sometimes many thousand) alternative interpretations of phylogenetic relationships are often equally parsimonious. Comparative analyses that use phylogenies should therefore test the sensitivity of their conclusions to the use of alternative phylogenetic hypotheses (Donoghue & Ackerly, this volume). At the moment this is a very arduous thing to do for large phylogenies, which is why we were unable to follow our own advice in the analysis of alkaloids, tannins and apparency, though improvements in software should soon make sensitivity analysis of this kind a practicality.

A concern often voiced by ecologists encountering PIC for the first time is that it is 'very wasteful of data' because there are many fewer contrasts than species in most datasets when binary variables such as annual/perennial are being analysed (the Abrahamson dataset discussed above is a typical example). The answer, seldom seen as helpful, is the point already made in the Introduction; that the degrees of freedom in TIPS analyses are inflated by pseudoreplication. A more helpful answer to the problem is the advice that datasets should be assembled with trait contrasts in mind so as to minimize the degree of redundancy, though of course one can only do this if the binary traits are known for a large number of species in advance; fortunately this is often the case.

Gittleman & Luh (1992) wisely suggest that before PIC is used in a comparative study, summary statistics on the data (e.g. nested ANOVA) should be used to determine whether phylogenetic effects are present for the traits of interest. If the influence of common descent is low (i.e. a high proportion of trait variance is found among species), then in the absence of a phylogeny for the group TIPS (or the equivalent at some higher taxonomic level) might be a reliable comparative method to use (e.g. Peat & Fitter 1994).

The problem of Type I error in TIPS and the fact that most of the ecological patterns observed to date have been identified using this method could be said to leave comparative plant ecology in the same state as a novel by Iris Murdoch; there are too many characters with too many unresolved relationships between them. The resolution of plant phylogeny promises also to resolve our picture of plant life history in all its aspects.

REFERENCES

Abrahamson, W. G. 1979 Patterns of resource allocation in wildflower populations of fields and woods. *Am. J. Bot.* **66**, 71–79.

Baker, H. G. 1972 Seed weight in relation to envionmental conditions in California. *Ecology* **53**, 997–1010.

Berenbaum, M. R. 1995 The chemistry of defense – theory and practice. *Proc. natn. Acad. Sci. U.S.A.* **92**, 2–8.

Bousquet, J., Strauss, S. H., Doerksen, A. H. & Price, R. A. 1992*a* Extensive variation in evolutionary rate of *rbc*L gene-sequences among seed plants. *Proc. natn. Acad. Sci. U.S.A.* **89**, 7844–7848.

Bousquet, J., Strauss, S. H. & Li, P. 1992*b* Complete congruence between morphological and *rbc*L-based molecular phylogenies in birches and related species (Betulaceae). *Molec. Biol. Evol.* **9**, 1076–1088.

Bremer, B. & Struwe, L. 1992 Phylogeny of the Rubiaceae and the Loganiaceae-congruence or conflict between morphological and molecular-data. *Am. J. Bot.* **79**, 1171–1184.

Bruneau, A., Dickson, E. E. & Knapp, S. 1995 Congruence of chloroplast DNA restriction site characters with morphological and isozyme data in *Solanum* sect lasiocarpa. *Can. J. Bot.* **73**, 1151–1167.

Chase, M. W., *et al.* (41 others) 1993 Phylogenetics of seed plants – an analysis of nucleotide-sequences from the plastid gene *rbc*L. *Annls Missouri Botanical Garden* **80**, 528–580.

Coley, P. D., Bryant, J. P. & Chapin, F. S. I. 1985 Resource availability and plant antiherbivore defense. *Science* **230**, 895–899.

Eggleton, P. & Vane-Wright, R. I. 1994 Some principles of phylogenetics and their implications for comparative

biology. In *Phylogenetics and ecology* (ed. P. Eggleton & R. I. Vane-Wright), pp. 345–366. London: Academic Press.

Feeny, P. P. 1976 Plant apparency and chemical defense. *Recent Adv. Phytochemistry* **10**, 1–40.

Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Naturalist* **125**, 1–15.

Foster, S. A. & Janson, C. H. 1985 The relationship between seed size and establishment conditions in tropical woody plants. *Ecology* **66**, 773–780.

Frascaria, N., Maggia, L., Michaud, M. & Bousquet, J. 1993 The *rbc*L gene sequence from chestnut indicates a slow rate of evolution in the Fagaceae. *Genome* **36**, 668–671.

Gittleman, J. L. & Luh, H. K. 1992 On comparing comparative methods. *A. Rev. Ecol. Syst.* **23**, 383–404.

Hancock, J. F. & Pritts, M. P. 1987 Does reproductive effort vary across different life forms and seral environments? A review of the literature. *Bull. Torry Bot. Club* **114**, 53–59.

Harvey, P. H. 1996 Phylogenies for ecologists. *J. Anim. Ecol.* **65**, 255–263.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology*. Oxford University Press.

Harvey, P. H., Read, A. F. & Nee, S. 1995 Why ecologists need to be phylogenetically challenged. *J. Ecol.* **83**, 535–536.

Jansen, R. K., Holsinger, K. E., Michaels, H. J. & Palmer, J. D. 1990 Phylogenetic analysis of chloroplast DNA restriction site data at higher taxonomic levels: An example from the Asteraceae. *Evolution* **44**, 2089–2105.

Kelly, C. K. 1995 Seed size in tropical trees: A comparative study of factors affecting seed size in Peruvian angiosperms. *Oecologia* **102**, 377–388.

Kelly, C. K. & Beerling, D. J. 1995 Plant life form, stomatal density and taxonomic relatedness: a reanalysis of Salisbury (1927). *Func. Ecol.* **9**, 422–431.

Kelly, C. K. & Purvis, A. 1993 Seed size and establishment conditions in tropical trees – on the use of taxonomic relatedness in determining ecological patterns. *Oecologia* **94**, 356–360.

Kelly, C. K. & Woodward, F. I. 1995 Ecological correlates of carbon isotope composition of leaves: a comparative analysis testing for the effects of temperature, CO_2 and O_2 partial pressures and taxonomic relatedness on delta C-13. *J. Ecol.* **83**, 509–515.

Kubitzki, K. & Ziburski, A. 1994 Seed Dispersal in Flood Plain Forests of Amazonia. *Biotropica* **26**, 30–43.

Levin, D. A. 1976 Alkaloid-bearing pants: an ecogeographic perspective. *Am. Nat.* **110**, 261–284.

Mabberley, D. J. 1987 *The plant book*. Cambridge University Press.

Martins, E. P. & Garland, T. 1991 Phylogenetic analyses of the correlated evolution of continuous characters – a simulation study. *Evolution* **45**, 534–557.

Miles, D. B. & Dunham, A. E. 1993 Historical perspectives in ecology and evolutionary biology – the use of phylogenetic comparative analyses. *A. Rev. Ecol. Syst.* **24**, 587–619.

Mole, S. 1993 The systematic distribution of tannins in the leaves of angiosperms – a tool for ecological studies. *Biochem. Syst. Ecol.* **21**, 833–846.

Patterson, C., Williams, D. M. & Humphries, C. J. 1993 Congruence between molecular and morphological phylogenies. *A. Rev. Ecol. Syst.* **24**, 153–188.

Peat, H. J. & Fitter, A. H. 1994 Comparative analyses of ecological characteristics of British angiosperms. *Biol. Rev. Camb. Phil. Soc.* **69**, 95–115.

Purvis, A., Gittleman, J. L. & Luh, H. K. 1994 Truth or consequences – effects of phylogenetic accuracy on 2 comparative methods. *J. theor. Biol.* **167**, 293–300.

Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comp. Appls Biosci.* **11**, 247–251.

Rees, M. 1995 EC-PC comparative analyses? *J. Ecol.* **83**, 891–892.

Rice, K. A., Donoghue, M. J. & Olmstead, R. G. 1995 A reanalysis of the large *rbc*L dataset. *Am. J. Bot.* **82** (Supplement), 157–158.

Rodman, J., Price, R. A., Karol, K., Conti, E., Sytsma, K. J. & Palmer, J. D. 1993 Nucleotide-sequences of the *rbc*L gene indicate monophyly of mustard oil plants. *Annls Missouri Botanical Garden* **80**, 686–699.

Salisbury, E. J. 1942 *The reproductive capacity of plants*. London: G.Bell & Sons.

Westoby, M., Leishman, M. R. & Lord, J. M. 1995 On misinterpreting the 'phylogenetic correction'. *J. Ecol.* **83**, 531–534.

Willson, M. F. 1983 *Plant reproductive ecology*. New York & Chichester: Wiley & Sons.

Wilson, A. M. & Thompson, K. 1989 A comparative study of reproductive allocation in 40 British grasses. *Func. Ecol.* **3**, 297–302.