Phylogenetic analysis of the ecological correlates of dioecy in angiosperms

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Abstract

We report on a phylogenetic analysis of correlations between the occurrence of dioecy and several ecological and life-history attributes: tropical distribution, woody growth form, abiotic pollination, small inconspicuous flowers and inflorescences, many-flowered inflorescences and fleshy fruits. Various hypotheses have been proposed to explain why associations occur between dioecy and several of these attributes, yet most assume that dioecy originates more often in clades with these traits than in clades with alternative character states. To investigate correlations between dioecy and these attributes, and to provide insights into the potential evolutionary pathways that have led to these associations, we assigned states of these traits to genera on a large-scale molecular phylogeny of the angiosperms; we then used maximum-likelihood analysis to analyse the presence of correlations and the sequence of acquisition of traits. Phylogenetic analysis revealed correlations between dioecy and six of the seven attributes; only many-flowered inflorescences exhibiting no association with the dioecious condition. The particular correlations that were revealed and the strength of the association differed among the three main monophyletic groups of angiosperms (Rosids, Asterids, and Eumagnoliids). Our analysis provided no general support for the hypothesis that dioecy is more likely to evolve in lineages already possessing the seven attributes we considered. Further analysis of the intercorrelations of the seven attributes provided evidence for non-independence between some of the traits, implying that functional associations among these traits have influenced the ecology and evolution of dioecious species.

Introduction

A principal goal of comparative biology is to determine the presence of correlations between morphological and ecological characters to gain insight into the evolution and adaptive significance of organismal traits (Harvey & Pagel, 1991; Silvertown *et al.*, 1997; Armbruster, 2002; Patterson & Givnish, 2002). This approach has been used in flowering plants to understand the evolution of separate sexes (dioecy) from hermaphroditism (reviewed in Sakai & Weller, 1999). Dioecy is a relatively rare sexual system occurring in approximately 7–10% of angiosperm species, yet it occurs in close to half of all flowering plant families (Renner & Ricklefs, 1995). This scattered phylogenetic distribution of dioecy among diverse families has stimulated workers to seek associations between sexual dimorphism and diverse ecological and life-history attributes (Givnish, 1980; Thomson & Brunet, 1990; Ibarra-Manriquez & Oyama, 1992). Several correlates of dioecy have been commonly reported in the literature, including: tropical distributions (Bawa, 1980), woody growth form (Fox, 1985), abiotic pollination (Renner & Ricklefs, 1995; Sakai *et al.*, 1997), small, inconspicuous, white or green–yellow flowers (Bawa & Opler, 1975; Bawa, 1980) and fleshy fruits (Muenchow, 1987). However, these associations were largely shown in studies that were either limited to local floras or particular taxonomic groups and/or did not control for the phylogenetic relationships among dioecious taxa.

Few studies have attempted to address correlations in worldwide dioecious angiosperms, and those to date

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(Renner & Ricklefs, 1995) have minimized the nonindependence of the data by analysing the presence of dioecy at higher taxonomic levels (i.e. genus and/or family level). As families and genera can often share traits as a result of common ancestry, many have argued that mapping traits on to phylogenetic trees is our best option for testing correlated evolution without bias from phylogenetic constraint (Felsenstein, 1985; Donoghue, 1989; Sanderson & Donoghue, 1996; Sakai *et al.*, 1997; Dodd *et al.*, 1999; Weiblen *et al.*, 2000). 'Correcting for phylogeny' may be particularly important when analysing dioecious angiosperms. This is because dioecious clades, although species-poor compared with cosexual sister-clades (Heilbuth, 2000), are more species-rich when associated with particular traits such as fleshy fruits (Donoghue, 1989), tropical distribution, woody growth form and inconspicuous flowers (J. C. Vamosi & S. M. Vamosi, unpublished data). If clades exhibiting contrasting traits experience differential diversification rates, nonphylogenetic tests seeking correlations will have a higher chance of finding spurious evidence of correlated evolution when such associations do not exist (Felsenstein, 1985).

Does the shift from hermaphroditism to dioecy occur before or after changes in geographical distribution, growth form, flower and inflorescence size, or fruit type? Most current theories assert that dioecy evolves more often in lineages that already possess these attributes (reviewed in Sakai & Weller, 1999). Indeed, several functional hypotheses have been formulated to explain the proposed order of historical events. For example, it has been suggested that low allocation to attractive structures (Charlesworth & Charlesworth, 1987) or heavy investment in fleshy fruits (Bawa, 1980; Givnish, 1980) are associated with dioecy because these traits give rise to more than linear gain curves with additional investment by an individual in either female or male function. This pattern is believed to favour the invasion of unisexual plants into cosexual populations (Charnov, 1982). It has also been suggested that the correlation between dioecy and small flowers results indirectly from an underlying correlation between small flowers and many flowers per inflorescence. Many-flowered inflorescences may lead to higher geitonogamous selfing resulting in strong inbreeding depression and selection for unisexuality (Barrett, 1984; Thomson *et al.*, 1989; Webb, 1999; de Jong, 2000). Arguments invoking inbreeding depression as the main selective force have also been proposed to explain why dioecy is correlated with woody growth form and large plant size (Bawa, 1980; Lloyd, 1982) and with abiotic pollination (Charlesworth, 1993). Unfortunately, little empirical evidence supports the idea that dioecy evolves subsequent to the evolution of any of these traits despite the abundance of adaptive hypotheses (Donoghue, 1989; Charlesworth, 1993; Sakai *et al.*, 1997).

The recent advent of phylogenetic comparative methods provides an opportunity for the extensive testing of correlations among life-history traits and ecology and investigation of the evolutionary history of these associations (Silvertown *et al.*, 1997). For example, the association between open habitats and floral form within the order Liliales was recently investigated by Patterson & Givnish (2002). They distinguished between two potential hypotheses that could explain trait correlations in this group: *concerted convergence*, where a trait is more likely to evolve in a lineage possessing another trait (as is often proposed for the origin of dioecy), and *phylogenetic niche conservatism*, where a trait is more likely to be maintained by selection when it is associated with another trait. Armbruster (2002) has further extended these ideas by distinguishing a third possibility where two traits evolve simultaneously on the same branch either from indirect selection of a third trait or due to pleiotropy between the two traits.

Using a large-scale molecular phylogeny of the angiosperms (Soltis *et al.*, 1999), we assigned to genera binary states for the following eight characters: sexual system (dioecious or nondioecious), distribution (tropical or temperate), growth form (woody or herbaceous), pollination (biotic or abiotic), flower type, inflorescence type (showy or inconspicuous), number of flowers per inflorescence (many-flowered or few-flowered) and fruit type (fleshy or dry). We then used this phylogenetic information to differentiate between the above hypotheses with regards to dioecy (Fig. 1). This analysis allowed us to gain insight into three main questions: (1) Taking phylogenetic relationships into account, is there evidence for the existence of correlations between dioecy and these seven traits? (2) What are the most likely evolutionary pathways leading to associations between dioecy and these traits, i.e. does dioecy commonly evolve in lineages with these traits or is the opposite order of events more common? (3) What evidence is there for intercorrelations among the traits examined? The presence of intercorrelations between the traits may give insight into which, if any, of the traits are likely to be functionally correlated with dioecy and which traits are correlated with dioecy because of other indirect associations. This, in turn, can guide investigations into how dioecy evolves and is maintained.

Methods

Character states of genera

We assigned the eight characters to the genera included on the shortest tree of the angiosperm phylogeny of Soltis *et al.* (1999). We analysed three major monophyletic taxa of the angiosperms (Rosids, Asterids, and Eumagnoliids), together making up 491 of the 560 genera in the phylogeny, separately due to memory constraints of the program. We coded all traits as binary

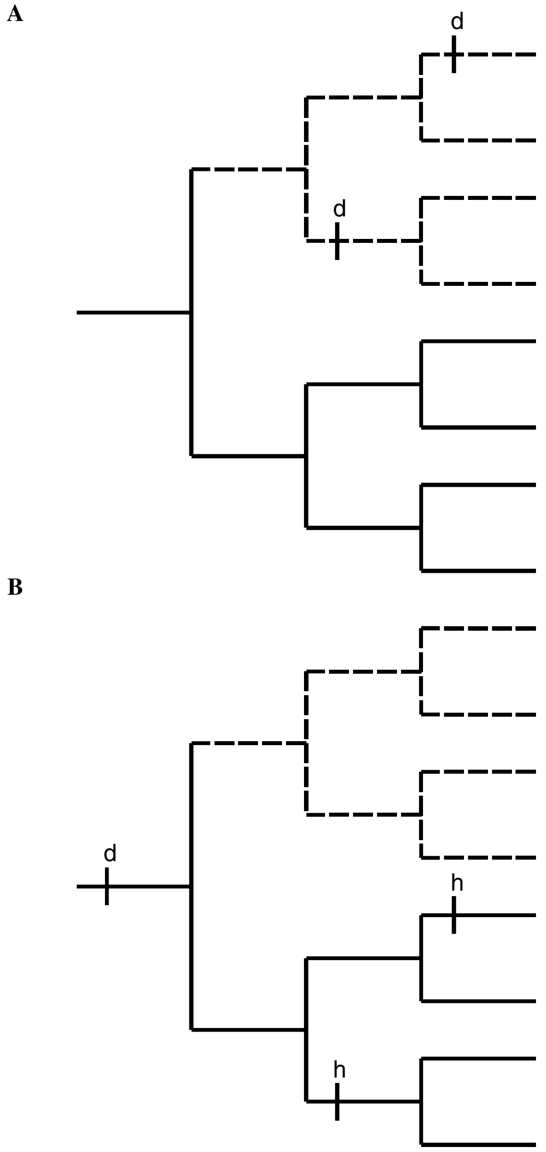


Fig. 1 A certain trait [e.g. dioecy (d) vs. hermaphroditism (h)] may evolve more often (A) or be more likely to be maintained (B) upon a background of another trait (e.g. dotted lines represent clade with fleshy fruits). Alternatively, the two traits may evolve in concert along a single branch (not shown), in which case the order of evolutionary events may be obscured.

and therefore genera were coded according to the state of the trait in the majority (≥50%) of its members (a full dataset of how all genera were coded is available from the first author). We determined sexual system from Takhtajan (1997), Mabberley (1997), and the database generously provided by Susanne Renner (University of Missouri), used in Renner & Ricklefs (1995). Based on these sources, 56 of 491 genera (~ 11%) had a majority (≥50%) of species that were dioecious. This proportion of dioecy is similar to estimates in the worldwide angiosperm flora (7–10%; Yampolsky & Yampolsky, 1922; Renner & Ricklefs, 1995; Weiblen *et al.*, 2000).

We obtained information regarding the geographical distribution, growth form, pollination system, flower and inflorescence type and fruit type for each genus from diverse literature sources including Bentham & Hooker (1867), Hutchinson (1964), Takhtajan (1997), Mabberley (1997) and various sites on the worldwide web (list of sources available from the first author). We coded genera with tropical or sub-tropical distributions as tropical, and trees, shrubs and lianas as woody. When sources did not report the presence of either fleshy or dry fruits, we coded fruit types such as drupes and berries as fleshy fruits, and capsules and samaras as dry fruits. Although some dry fruits such as nuts are biotically dispersed, a trait that has also been correlated with dioecy (Renner & Ricklefs, 1995), we chose to analyse the presence of fleshy fruits to have our work aligned in such a way as to better test the Givnish–Bawa hypothesis and the work of Donoghue (1989). When information regarding showiness of flower could not be found, we determined the status of the trait by examining pictures and drawings of representatives of the genus. We scored flowers judged to be white or yellow–green and smaller than 10-mm long as inconspicuous, even though these inconspicuous flowers may group together into showy inflorescences.

As the individual flower is not always the best measure of a plant's allocation to attractive features and the inflorescence in many cases represents the functional unit of reproduction (see Harder & Barrett, 1996), we also investigated the numbers of flowers per inflorescence and the showiness of inflorescences. To divide the numbers of flowers per inflorescence into a binary trait, we coded the genus as having 'many' flowers per inflorescence if it had >10 flowers per inflorescence and 'few' if it had fewer than 10 flowers. We obtained information regarding flower number per inflorescence by either finding such information on databases (e.g. Bertin & Newman, 1993) and floras or gaining our own estimates by counting the number of flowers per inflorescence in photographs found on the web and in the literature (list of sources available from first author). We coded all genera that were showy at the single-flower level as having showy inflorescences, and all genera that were coded as inconspicuous at the single-flower level and had <10 flowers per inflorescence as having inconspicuous inflorescences. We coded inconspicuously flowered genera that had high numbers of flowers per inflorescence as showy unless they were apetalous and asepalous (had no perianth) or were described as having green petals and sepals. The proportion of genera scored in each state for each of the eight traits is given for Rosids, Asterids, and Eumagnoliids in Table 1.

States for fruit type, growth habit, distribution, pollination, and inflorescence size were fairly uniform within genera (i.e. the source of botanical information generally

Table 1 The proportion of taxa in the Soltis *et al.* (1999) phylogeny with dioecy and the seven traits investigated in this study.

	N	Dioecy	Fleshy fruits	Woody	Tropical	Inconspicuous flowers	Inconspicuous inflorescences	Many-flowered inflorescences	Abiotic pollination
Rosids	172	9.9	34.9	80.8	71.5	54.1	37.8	48.8	14.0
Asterids	181	9.9	35.9	54.7	74.0	45.3	17.7	45.3	3.3
Eumagnoliids	138	15.2	49.3	39.9	76.1	50.7	28.3	47.1	9.4
Combined	491	11.4	39.3	59.7	73.7	49.9	27.7	47.0	8.8

quoted that the entire, or most, of the species in a genus have fleshy fruit, woody growth form, etc.). However, the states of flower size and the number of flowers per inflorescence were far more variable, and the accuracy of our trait estimates will be poorest in these categories. Typically (~ 90% of the genera), it was straightforward to code the genus as having either less than, or more than, 10 flowers per inflorescence and single flowers that were less than, or greater than, 10 mm as the genus had species that were clearly in one category or the other (e.g. all species of *Salix* have very small flowers and far more than 10 flowers per inflorescence). However, in a few genera these traits were quite variable or appeared to have approximately 10 flowers per inflorescence (or the flowers were ~ 10-mm wide). To test for the robustness of our results in the face of potential inaccuracies in the dataset, we coded the most ambiguous genera for the flower size trait (5, 5, and 3 genera in Rosids, Asterids, and Eumagnoliids, respectively) and number of flowers per inflorescence trait (5, 8, and 2 genera in Rosids, Asterids, and Eumagnoliids, respectively) with our best estimate of the state based on the majority of species in the genus. We then re-ran the program testing the correlations between dioecy and these two traits assuming that all of these best estimates were in error. As our results changed very little, we conclude that our results are fairly robust to minor changes in the dataset, and report only the results using our best estimates.

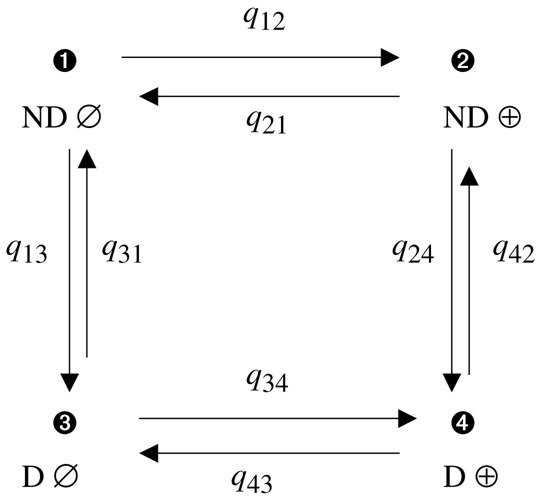


Fig. 2 Two sexual systems [Dioecious (D) or Nondioecious (ND)] and two states [present (⊕) or absent (∅)] for a putatively correlated character of interest (e.g. woody growth form) gives four different combinations of characters. The probability of observing combinations 1–4 depends on the transition rates (q_{ij}) between states 1 and 2, states 1 and 3, states 2 and 4, and states 3 and 4 in either direction. It is assumed that only one transition can occur at a time and so the transition rates of q_{14}, q_{23}, q_{41}, $q_{32} = 0$. Dioecy and the trait can become positively correlated if one or more of the following transition rate inequalities are true: $q_{12} < q_{34}$; $q_{13} < q_{24}$; $q_{42} < q_{31}$; $q_{43} < q_{21}$.

Tests of correlated evolution

The maximum-likelihood program Discrete (Pagel, 1994, 1997) enables investigation of both the presence and cause of trait correlations by comparing the rate of transition between the four combinations of any two binary traits (Fig. 2). It differs from other methods of inferring correlation between traits because the ancestral states of individual nodes of a phylogenetic tree are not estimated. Using the Discrete program (Pagel, 1994), the probability of observing specified character states for sexual system (X) and the correlated trait (Y) at the tips of a phylogeny is estimated given transition rates (q) for each of the traits (see Fig. 2). To determine if dioecy is correlated with a trait, it is necessary to compare the likelihood estimate obtained when it is assumed that the evolution of the two traits (e.g. dioecy and showy flowers) occurs independently, L(I), to the likelihood estimate obtained when the transition rates of the traits are allowed to be nonindependent, L(D). That is, L(I) is obtained by assuming $q_{12} = q_{34}$, $q_{13} = q_{24}$, $q_{42} = q_{31}$ and $q_{43} = q_{21}$, whereas L(D) is obtained without these constraints. Likelihood ratios, which approximately follow a chi-square distribution (Pagel, 1994), indicate correlated evolution if L(D) is significantly greater than L(I) tested by comparing $-2[\mathrm{L(D)} - \mathrm{L(I)}]$ to a chi-square distribution with 4 d.f.

This model can be used to test specific hypotheses about the nature of the correlated changes in X and Y to distinguish if a more frequent sequence of acquisition of the traits occurs (the contingent-change test). Likelihood estimates obtained with the unrestricted model [L(D)] can be compared with the likelihood estimates obtained with a model that restricts two of the rates (e.g. restricting the rate that dioecious and nondioecious clades evolve showy flowers to be equal ($q_{12} = q_{34}$; see Fig. 2). The log-likelihood ratio, $-2[\mathrm{L(D)} - \mathrm{L}(q_{12} = q_{34})]$, then approximately follows a chi-square distribution with 1 d.f. For each trait, we calculated contingent-change tests to distinguish between the concerted evolution and phylogenetic niche conservatism hypotheses. Furthermore, if $q_{24} + q_{34} \gg q_{12} + q_{13}$ or $q_{21} + q_{31} \gg q_{42} + q_{43}$ (see Fig. 2), this indicates that the two traits will tend to change one right after the other, either indicating that the traits are linked through pleiotropy, indirect selection via a third unknown trait, or by extremely strong concerted evolution (Armbruster, 2002). Because of large ratios between large and small branch lengths in the Soltis *et al.* (1999) phylogeny, we employed the scaling parameter of Discrete, as suggested by Pagel (1994). This scaling parameter reduces the ratio of the largest to shortest branches and makes the maximum likelihood (ML) estimates more stable (Pagel, 1994). As the Discrete program continues its reiterative function until it calculates two very close ML estimates in a row, there is some variability in the largest ML estimate reported. Therefore, we obtained three likelihood estimates for L(D), L(I), and all of the restricted likelihoods, and took the largest ML estimate obtained in each case.

We tested for interrelations between the traits in much the same way. We did not test two of the correlations involving floral display because these were interdependent by definition. First, the correlation between inconspicuous flowers and inconspicuous inflorescences was not examined because there would obviously be a positive correlation between these two traits due to the fact that a showy solitary flower was automatically coded as having a showy inflorescence. Hence, there was a paucity of clades having a showy flower, inconspicuous inflorescence combination, which would alter the transition rates in nonnormal ways. Second, we did not test for a correlation between inconspicuous inflorescences and many-flowered inflorescences. This is because a negative correlation was inevitable because all inflorescences with >10 flowers were automatically coded as showy (see above) unless they had no perianth, making a paucity of clades having a trait combination of many flowers and inconspicuous inflorescences.

Combining the probabilities from the different subsets (Rosids, Asterids, and Eumagnoliids) is straightforward using a likelihood approach. Log-likelihoods and d.f. combine additively, and a log-likelihood test can be performed on the sums of the log-likelihoods for L(I) and L(D) from Rosids, Asterids, and Eumagnoliids (Edwards, 1972). As we used every character trait combination in multiple tests (one test to determine whether a correlation was present and four tests to determine the cause of the correlation (yielding $n = 5$ tests in total), we adjusted the α-level using the sequential Bonferroni correction (Rice, 1989). With this correction, the test with the greatest significance level requires $P < 0.05/n$ in order to be significant, the second greatest requires $P < 0.05/(n - 1)$, the third requires $P < 0.05/(n - 2)$, and so on.

Results

Traits correlated with dioecy

The results of the maximum likelihood analysis (summarized in Table 2) indicate that when phylogenetic relationships are taken into account, dioecy is correlated with tropical distributions, white or green–yellow inconspicuous flowers, and inconspicuous inflorescences in all three major groups of angiosperms ($P < 0.005$ in each). Dioecy is also correlated with fleshy fruits and the woody habit in the Eumagnoliids ($P = 0.003$ and $P < 0.001$, respectively) and the Asterids ($P = 0.015$ and $P < 0.001$, respectively). Dioecy is correlated with abiotic pollination in the Rosids and Asterids ($P < 0.001$ and $P < 0.01$, respectively). In none of the groups, however, was dioecy correlated with many-flowered inflorescences ($P > 0.10$ in each). Combining the probabilities obtained for the three major taxa resulted in highly significant correlations for all of the ecologically important attributes ($P = 0.005$) with the exception of many-flowered inflorescences (Table 3). These correlations remained highly significant after sequential Bonferroni correction with $n = 5$ (Rice, 1989).

Order of establishment of traits correlated with dioecy

By combining the probabilities obtained from the Rosids, Asterids, and Eumagnoliids, we found only two patterns that were significant at the level of $\alpha = 0.05$ (but not with Bonferroni correction with $n = 5$) and consistent amongst all three groups: (1) tropical distribution evolves more often in clades that are dioecious ($P = 0.023$); and (2) dioecy evolves more often in clades that have inconspicuous inflorescences ($P = 0.027$). Interestingly, there were several patterns that differed between the major clades of angiosperms, even though none are significant after segmential Bonferroni correction. For example, in the Eumagnoliids, the correlation with fleshy fruits arises, in part, because fleshy fruits are more likely to evolve in dioecious clades ($q_{12} < q_{34}$; $P = 0.040$; contingent-change test). In contrast, in the Asterids the reverse sequence was evident with dioecy originating more commonly in clades that already possess fleshy fruits ($q_{13} < q_{24}$; $P = 0.028$; contingent-change test). Dioecy also appears to evolve more commonly in tropical clades within the Asterids ($q_{13} < q_{24}$; $P = 0.025$). In the Rosids, dioecy originated more often in clades with many-flowered inflorescences ($P = 0.040$) although this pattern was not strong enough to cause a correlation between dioecy and many-flowered inflorescences ($P = 0.107$). A trend that was consistent among all three

Table 2 Associations between dioecy and ecological traits. Summary of results from combining data from all three major groups (Rosids, Asterids, and Eumagnoliids) using the Discrete program and the phylogeny of Soltis *et al.* (1999). Entries above the diagonal reflect whether correlated evolution was detected whereas entries below the diagonal indicate the evolutionary pathway determined to be important in causing the association between the two traits. Abbreviations are as follows: '+' = positive correlation; NS = not significant; n/a = test not performed because no correlation found; D = dioecy; ND = nondioecy; F = fleshy fruit; Dr = dry fruit; W = woody; H = herbaceous; Tr = Tropical; Te = Temperate; I = Inconspicuous flowers; H = high numbers of flowers per inflorescence. The entry (Dr,W ⇒ F,W), e.g. should be read as 'Fleshy fruits evolve more often in woody clades'.

	Dioecy	Fleshy fruit	Woody habit	Tropical distribution	Inconspicuous flowers	Inconspicuous inflorescences	Many-flowered inflorescences	Abiotic pollination
Dioecy	–	+	+	+	+	+	NS	+
Fleshy fruit	NS	–	+	+	+	+	NS	NS
Woody habit	NS	Dr,W ⇒ F,W Dr,W ⇒ Dr,H	–	NS	NS	NS	NS	NS
Tropical distribution	D,Te ⇒ D,Tr	Dr,Tr ⇒ Dr,Te	n/a	–	NS	NS	NS	NS
Inconspicuous flowers	NS	I,Dr ⇒ I,F	n/a	n/a	–	Not examined	+	+
Inconspicuous inflorescences	ND,I ⇒ D,I	I,Dr ⇒ I,F	n/a	n/a	n/a	–	Not examined	+
Many-flowered Inflorescences	NS	NS	n/a	n/a	NS	NS	–	+
Abiotic pollination	NS	NS	n/a	n/a	NS	NS	NS	–

Table 3 Likelihood-Ratio values for tests of the presence and cause of proposed correlated evolution between dioecy and a range of ecological traits. In any test of correlated evolution there are four possible causes, which are not mutually exclusive. Boldface indicates that the transition rates were such that they would cause a negative correlation between dioecy and the trait in question (e.g. dioecy appears to evolve more often in clades with dry fruit in the Rosids), but these rate ratios do not have a very strong effect. Negative log-likelihood ratios are theoretically impossible but occur occasionally due to the stochastic nature of the Discrete program [L(D) is the ML where all rates are unrestricted and should therefore always have the higher likelihood compared to tests where restrictions are placed on the rates; see Methods].

	Group	Fleshy fruit	Woody habit	Tropical distribution	Inconspicuous flowers	Inconspicuous inflorescences	Many-flowered inflorescences	Abiotic pollination
Correlated evolution	Rosids	2.4	0	17.6**	37.2***	28.4***	3.8	26.4***
	Asterids	12.4*	19.2***	14.8*	8.8	38.8***	3.4	13.4*
	Eumagnoliids	15.8**	85.0***	25.0***	19.2***	10.7***	3.2	0.2
	All	**	***	***	***	***	NS	***
Dioecy evolves more often in clades with trait	Rosids	**−0.8**	−0.4	−0.2	−0.2	5.4*	4.2*	0.8
	Asterids	4.8*	3.4	5.0*	1.2	1.6	0.6	1.4
	Eumagnoliids	**−0.2**	**0.2**	**1.2**	2.4	2.2	0.8	1.6
	All	NS	NS	NS	NS	*	NS	NS
Trait evolves more often in dioecious clades	Rosids	3.2	**1.0**	2.8	0.6	**1.2**	0	3.4
	Asterids	**−0.8**	1.4	0.8	0.2	1.0	2.0	**0.6**
	Eumagnoliids	4.2*	2.2	5.2*	1.2	2.4	0	**0**
	All	NS	NS	*	NS	NS	NS	NS
Dioecy is lost more often in clades with opposite trait	Rosids	0.4	**1.0**	−0.4	2.4	**0.8**	**1.6**	**0.6**
	Asterids	**0.2**	**0.2**	**2.2**	**0**	1.4	1.8	1.4
	Eumagnoliids	**0.8**	1.8	**3.0**	**0**	1.4	2.2	0.6
	All	NS	NS	NS	NS	NS	NS	NS
Opposite trait evolves more often in cosexual clades	Rosids	**1.4**	0.8	**2.0**	1.2	0.6	**2.8**	1.0
	Asterids	0.4	**−0.2**	0.4	0	**1.4**	**0.8**	0.6
	Eumagnoliids	**0**	−1.2	**0.8**	**0.4**	**2.4**	**1.8**	**2.2**
	All	NS	NS	NS	NS	NS	NS	NS

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; NS = not significant.

groups, although not significant, involved few-flowered inflorescences evolving more often in dioecious clades. This pattern may have contributed to the lack of correlation between dioecy and many-flowered inflorescences reported in the preceding section.

There were several evolutionary transitions that commonly occurred together on the same branch of the phylogeny, thus obscuring our ability to trace the history of the correlates of dioecy (see Appendix 1). Pleiotropy, indirect selection, or extremely tight contingent evolution was indicated (i.e. $q_{24} + q_{34} \gg q_{12} + q_{13}$ and/or $q_{21} + q_{31} \gg q_{42} + q_{43}$) as a potential cause of the correlation between dioecy and the following three traits: inconspicuous flowers (Eumagnoliids and Rosids, especially), inconspicuous inflorescences (Eumagnoliids and Asterids) and abiotic pollination (Asterids). This result indicates that transitions in a trait are strongly influenced by the presence of the other (or, alternatively, the presence of an unknown third trait is influencing transitions in both), but it is not possible to deduce which is the independent and which is the dependent evolutionary change.

Associations between correlated traits of dioecy

Many of the correlates of dioecy do not appear to be independent (Table 4). Averaging over all three angiosperm lineages, fleshy fruits are positively correlated with the woody habit ($P < 0.001$), tropical distribution ($P = 0.010$), inconspicuous flowers ($P = 0.014$) and inconspicuous inflorescences ($P = 0.024$). Not surprisingly, both inconspicuous flowers and inflorescences were correlated with abiotic pollination ($P < 0.001$, for both). Finally, inconspicuous flowers were also correlated with many-flowered inflorescences ($P < 0.001$). Although not common to all groups, a negative correlation was also evident between abiotic pollination and tropical distribution in the Asterids ($P = 0.015$). With the sequential Bonferroni correction, only the correlations between fleshy fruit and woody habit, between inconspicuous floral display (at the level of flower and inflorescence) and abiotic pollination, and between inconspicuous flowers and many-flowered inflorescences, would be considered truly significant.

Tracing the evolutionary histories that have given rise to the trait correlations in fleshy-fruited species was informative. The correlation between fleshy fruit and woody habit is caused largely by fleshy fruits evolving more often in woody lineages ($P < 0.001$), with a marginally significant contribution coming from herbaceousness evolving more often in dry fruited rather than fleshy fruited clades ($P = 0.020$). The correlation between fleshy fruit and tropical distribution appears to be caused primarily by temperate distribution evolving more often in dry fruited clades ($P = 0.049$). The correlation between fleshy fruits and both inconspicuous flowers and inflorescences appears to result from fleshy fruits evolving more often in clades with low floral display ($P < 0.001$ and $P = 0.042$, for flowers and inflorescences respectively). The explanations for the correlations between inconspicuous flowers per inflorescences and abiotic pollination, and the correlation between inconspicuous flowers and many-flowered inflorescences are unclear, but may be because the two traits commonly changed in concert on the same branch ($q_{21} + q_{31} \gg q_{42} + q_{43}$ and/or $q_{34} + q_{24} \gg q_{12} + q_{13}$ in all cases, data not shown), suggesting that their evolution is too tightly linked to tease apart with the broad-scale analysis used in this study.

Discussion

The results of our phylogenetic analysis of the ecological correlates of dioecy in flowering plants indicate that this sexual system is correlated with tropical distribution, woody growth form, abiotic pollination, inconspicuous flowers, inconspicuous inflorescences and fleshy fruits. Although several earlier studies found evidence for associations between dioecy and several of these traits (reviewed in Sakai & Weller, 1999), our analysis is the first to explicitly take into account the phylogenetic nonindependence of data used in such comparative surveys. By splitting the angiosperm genera included in our analysis into three monophyletic groups we also found evidence that several of these correlations were stronger among the Eumagnoliids than in the other two groups. This pattern was particularly evident for the correlations between dioecy and woody growth form and tropical distribution. Here we discuss some of the potential adaptive explanations for the associations that were revealed in our study and also consider some of the limitations of phylogenetic analyses of large data sets that may influence the interpretation of our results.

Dioecy and floral display

We found evidence that dioecy evolves more often in clades with inconspicuous inflorescences. Although a significant pattern (albeit not when a correction for multiple tests was used) was only found between dioecy and inflorescence showiness, the patterns observed between dioecy and inconspicuous flowers were qualitatively similar. These observations are consistent with the hypothesis that dioecy evolves in clades with a low floral display as proposed by Charnov *et al.* (1976), Charlesworth & Charlesworth (1987), and Charlesworth (1993) rather than the opposite order of events as argued by some (Kaplan & Mulcahy, 1971). Specifically, our result that dioecy evolves in clades that have inconspicuous floral displays provides support for the model proposed by Charlesworth & Charlesworth (1987) that females can more readily invade populations that allocate little to attraction, perhaps because low investment in flowers allows females to devote more resources to fruit (Bawa, 1980; Givnish, 1980; Richards, 1997). The correlation between dioecy and abiotic pollination was very strong, as was the correlation between abiotic pollination and both flower and inflorescence size. No particular sequence of evolutionary events was evident in our analysis of dioecy and pollination system. Future work involving phylogenies within clades that are variable in terms of pollination mode and sexual system (e.g. see Sakai *et al.*, 1997) are necessary to understand the

Table 4 Likelihood-ratio values testing for the presence of correlations among the ecological traits that are correlated with dioecy. In each set of four rows, first row = Rosids; second row = Asterids; third row = Eumagnoliids; fourth row = Combined test. Entries above the diagonal indicate whether a correlation was detected (negative correlations are depicted in boldface). The transition rates for any significant correlations are below the diagonal and are in the order of: first column = whether trait X evolves more often in a background of trait Y; second column = trait Y evolves more often upon a background of trait X; third column = trait X is lost less often in clades with trait Y; fourth column = trait Y is lost less often in clades with trait X. X = Column state. Y = Row state.

	Fleshy fruit				Woody habit	Tropical distribution	Inconspicuous flowers				Inconspicuous inflorescences				Many-flowered inflorescences	Abiotic pollination
Fleshy fruit	–				26.4***	6.2	12.0*				6.2				1.0	6.2
					8.4	10.2*	7.4				10.6*				4.6	0
					15.0*	9.8*	5.8				6.6				5.0	2.1
					***	*	*				*				NS	NS
Woody habit	6.2*	2.2	**3.0**	5.0*	–	−1.4	5.6				5.0				0	−2.4
	0.6	**2.2**	1.6	3.8		7.1	8.8				6.4				4.0	1.6
	9.8**	2.0	**0.6**	0.6		0.2	3.2				2.6				8.9	4.6
	***	NS	NS	*		NS	NS				NS				NS	NS
Tropical distribution	0.8	−0.2	**1.6**	0	N/A	–	−1.0				0.2				−0.2	1.2
	1.8	3.6	1.4	3.4			2.4				8.6				5.6	**12.4***
	0.4	0.4	**−0.2**	4.8*			2.4				0.4				4.8	−0.4
	NS	NS	NS	*			NS				NS				NS	NS
Inconspicuous flowers	11.6***	**−0.4**	**3.8**	1.6	N/A	N/A	–				Not examined				8.8	23.8***
	2.2	0.2	1.8	2.6											14.8*	10.8*
	4.0*	**0.2**	**1.0**	1.2											8.4	19.0***
	***	NS	NS	NS											**	***
Inconspicuous inflorescences	3.6	2.4	**2.0**	**−0.2**	N/A	N/A	N/A				–				Not examined	31.4***
	0.6	**0.2**	0.2	1.8												25.6***
	5.8*	0.2	1.6	**2.8**												20.0***
	*	NS	NS	NS												***
Many-flowered inflorescences	N/A				N/A	N/A	1.6	0.4	5.2*	**1.8**	N/A				–	6.8
							1.2	**0**	2.0	0.2						3.4
							1.0	0.6	−0.2	**1.4**						5.0
							NS	NS	NS	NS						NS
Abiotic pollination	N/A				N/A	N/A	**0.8**	**0.6**	2.4	3.2	1.2	**0.4**	3.6	**2.4**	N/A	–
							0.2	0	**−0.2**	**−0.2**	1.2	1.2	1.4	**1.4**		
							0.6	2.2	**0.4**	**0.4**	1.2	−2.6	1.0	0.4		
							NS	NS	NS	NS	NS	NS	NS	NS		

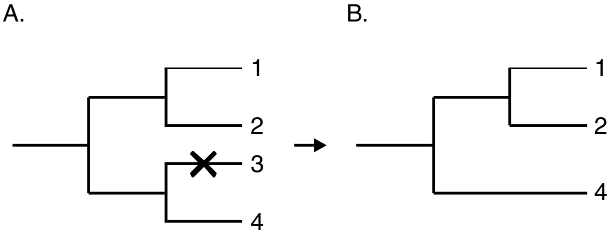
*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; NS = not significant; N/A = Not available because correlation was not significant.

processes responsible for the correlation between dioecy and abiotic pollination.

It has been argued that the correlation between dioecy and small flowers is due to an association between dioecy and many-flowered inflorescences (Thomson *et al.*, 1989). This could arise because of a trade-off between flower size and number with many-flowered inflorescences possessing small flowers (reviewed in Worley & Barrett, 2000). In theory, dioecy might be expected to evolve more readily in clades with many-flowered inflorescences because: (1) many-flowered inflorescences often present only a small proportion of their flowers at a time, resulting in a more than linear male gain curve and conditions favouring the evolution of dioecy (Thomson *et al.*, 1989); and/or (2) having many flowers per inflorescence causes more geitonogamous selfing, and hence favours the spread of unisexuals (Harder & Barrett, 1996). In our analysis, dioecy was found to evolve more often in lineages with higher numbers of flowers per inflorescence in the Rosids. However, we did not find a significant correlation between dioecy and many-flowered inflorescences, largely because there was a trend for few-flowered inflorescences to evolve in dioecious clades. This further points to the importance of the correlation between low floral display size and dioecy and refutes the idea that the correlation between small flowers and dioecy is only a by-product of a correlation between many-flowered inflorescences and small flowers.

Dioecy and tropical distribution

The correlation between dioecy and tropical distribution has often been reported and assumed to be an indirect correlation caused by associations between tropical distribution and fleshy fruits, woody growth form, and inconspicuous flowers (see Sakai & Weller, 1999). However, tropical distribution was found to be independently associated with dioecy at the family level by Renner & Ricklefs (1995). Our analysis found that the only other correlation with tropical distribution was with fleshy fruits and even this association disappeared after correction for multiple tests. This observation suggests that a direct causal link between tropical distribution and dioecy may exist. Perhaps even more intriguing is the result that tropical distribution evolves within dioecious lineages, rather than the opposite, more intuitive, order of events. Although speculative, we give below two potential explanations as to why such a pattern might occur.



	Before extinction	After extinction
q_{12}	0.26	0.22
q_{13}	0.59	<0.01
q_{21}	<0.01	0.28
q_{24}	0.69	1.57
q_{31}	0.74	12.65
q_{34}	<0.01	19.12
q_{42}	0.55	1.61
q_{43}	0.27	<0.01

Fig. 3 Simplified example in which differential extinction of certain clades can alter the transition rates in Discrete. Clades are represented as they were in this study: (1) nondioecious and temperate; (2) nondioecious and tropical; (3) dioecious and temperate; (4) dioecious and tropical. Extinction takes place more often in clades with combination 3, perhaps because the clades are maladapted to cold environments present after a cooling event. The phylogeny that we are able to analyse is obviously not the true phylogeny (A), but the one where extinction has already taken place (B). The extinction of clade 3 alters the transition rates obtained from Discrete (as shown in the table) by both reducing the rates at which a clade with combination 3 is formed (q_{13}, q_{43}), as well as escalating the rates at which clades with combination 3 become combination 1 or combination 4 (q_{31}, q_{34}). This alteration in rates can give the erroneous indication that dioecious clades become tropical more often ($q_{34} > q_{12}$) when, in fact, differential extinction is the true cause of why dioecy is more prevalent in the tropics.

One potential reason for the association between tropical distribution and dioecy is due to climate changes overtime. Fossil evidence indicates that more of the earth's surface was tropical in the past. Also there were a number of cooling events in geological time (Tiffney, 1985) leading to several angiosperm genera and families with disjunct [e.g. *Aralia* (Wen, 2000) and *Magnolia* (Azuma *et al.*, 2001)] and/or small (relict) distributions (e.g. *Bencomia* alliance; Helfgott *et al.*, 2000). If during periods of retraction of the tropical zone, dioecious clades left in areas that became temperate suffered extinction or range contraction more than nondioecious clades, this could lead to an apparent pattern of dioecy evolving first, and tropical climate after (see Fig. 3). As the most severe temperature fluctuations took place during the Eocene, a comparison of our results with results of analyses of clades that diverged after the Eocene (33 mya) may give some indication of how these warming and cooling periods affected the evolution of sexual systems. Dioecious clades may be more vulnerable to extinction in temperate climes perhaps because they are more susceptible to fluctuations in pollinators (Vamosi & Otto, 2002) and/or animal dispersers (Heilbuth *et al.*, 2001), which may accompany large fluctuations in temperature in temperate zones. A recent study has shown that tropical dioecious clades are more species-rich than temperate dioecious clades, whereas the difference in species richness between geographical regions was not as profound for nondioecious clades (J. C. Vamosi & S. M. Vamosi, unpublished data). This finding indicates that differential extinction of dioecious clades may be a factor in causing the correlation we observe between dioecy and tropical distribution.

A second potential reason for dioecious clades to migrate to tropical climes may lie with the observed correlation between dioecy and fleshy fruits. Fleshy fruits, which are often dispersed by birds, may increase the chance that a species successfully disperses over long distances. Dioecious clades have been quite successful at long-distance colonization among tropical environments [e.g. to Hawaiian islands (Sakai *et al.*, 1995); *Gaertnera* dispersal to south-east Asia (Malcomber, 2002)]. Dispersal may also have occurred from temperate areas towards tropical ones. Because dispersal is envisioned to occur more commonly in the opposite direction, however, i.e. with clades arising in tropical environments and migrating to temperate environments (Axelrod, 1959; Kalkman, 1988; Manos & Stanford, 2001), the involvement of climatic change in causing the pattern of dioecious clades invading tropical regions may be more plausible.

Intercorrelation among traits

We were able to detect several intercorrelations among traits that could influence the positive associations that we observed with dioecy. Fleshy fruits, for instance, were observed to have associations with a woody growth habit, most markedly, but also with tropical distribution, inconspicuous flowers and inconspicuous inflorescences. Furthermore, inconspicuous flowers and inconspicuous inflorescences were, not surprisingly, correlated with abiotic pollination, and, finally, inconspicuous flowers and abiotic pollination were both correlated with having many-flowered inflorescences. Despite the multitude of intercorrelations observed, not all of the correlations observed with dioecy can be explained by these intercorrelations. For instance, although a correlation was found between fleshy fruits and small flowers, dioecy was observed to be correlated with inconspicuous flowers but *not* fleshy fruits in the Rosids, and yet dioecy was correlated with fleshy fruits but *not* inconspicuous flowers in the Asterids. Determining which traits play a causal role in the evolution of dioecy could be undertaken by analysing clades in which variation exists in only one ecological trait. Such phylogenies are rare at the present time, but the potential exists for future studies on this topic.

The correlations found between several of the traits analysed in this study give insight into questions unrelated to the evolution of sexual systems. Our results are consistent with a recent phylogenetic study (Patterson & Givnish, 2002) that detected an association between fleshy fruits and inconspicuous flowers in connection with closed canopy habitats in the Liliales. However, some of our results are in contrast to the results of other studies that either used only local taxa and/or did not use a phylogenetic approach. Ibarra-Manriquez & Oyama (1992) found that larger fruits were correlated with showy flowers. Our results were also at odds with previous studies in two other respects. First, Ricklefs & Renner (1995) found a positive association between biotic pollination and woody growth form but we did not. Second, the expected association between abiotic pollination and temperate distribution was only detected in the Asterids, despite reports of limited wind pollination in tropical woody plants (Bawa *et al.*, 1990; Ricklefs & Renner, 1994). These differences may have been due to the phylogenetic correction used in this study. For instance, in the Rosids, many groups were abiotically pollinated and temperate (those in the families Tetramelaceae, Fagaceae, Juglandaceae, Myricaceae, etc.) but these families are clustered in one section of the angiosperm phylogeny. Previous studies that have compared families as independent units (Ricklefs & Renner, 1994; Renner & Ricklefs, 1995) minimized the effects of phylogeny without erasing its effects altogether.

Limitations of study and future directions

There are several difficulties in performing comparative analyses of large-scale phylogenies that are likely to influence the interpretations of the results we obtained. First, incomplete phylogenies may place a showy, cosexual genus as the sister genus to a small-flowered dioecious taxon. When few genera are present with only one of the traits, the order of acquisition of each trait is hard to infer. Second, it should be noted that our method of combining probabilities to find general patterns assumes that the three major clades are independent. When the Rosids, Asterids, and Eumagnoliids are examined separately, the Discrete program automatically assigns a null value to the root node. Admittedly, this is an over-simplification as the three clades are related to one another and the ancestral reconstruction of the root of one clade automatically affects the likelihood of the root of the other two clades. Given that the correlations reported in this study were observed to be strong and largely consistent within all three clades, it is unlikely that analysing all angiosperms together (were it possible) would alter our main results. Third, differential diversification rates need to be addressed as well. As pointed out by Schluter (2000), traits that confer decreased diversification (i.e. woody growth form, see Dodd *et al.*, 1999) may place those groups on the tips of long branches of a phylogeny. Because long-branch attraction towards an out-group is a problem in phylogenetic reconstruction, traits associated with low diversification may incorrectly appear to evolve first on the phylogeny with traits conferring high diversification evolving later (Schluter, 2000). Similarly, the more common state is also more likely to be reconstructed as evolving early when ancestral states are reconstructed via parsimony, although using Discrete should minimize this problem as all possible ancestral states are considered (Schluter *et al.*, 1997). Our analysis of dioecy suffers from both of these potential biases because dioecious lineages have been shown to experience decreased diversification compared to lineages with other sexual systems (Heilbuth, 2000) and was also the rare state in our analysis (only 11% of the genera). Nevertheless, having a tropical distribution is widely thought to lead to higher species richness (e.g. Gaston & Williams, 1996) and was the more common state in our analysis yet we still found evidence for the tropical trait to evolve after dioecy. There are no methods yet to measure and address these biases but, regardless, they should not affect our results on whether or not a correlation exists. However, our results regarding the sequence of acquisition of correlated traits should be interpreted with caution.

In the future it will be particularly important to examine detailed phylogenies of individual families and genera to ensure that the phylogeny is as complete as possible and that clade diversification differences are minimized. Moreover, performing tests on many smaller lineages may uncover the lineage-specificity of certain patterns. Our analysis, which divided the angiosperms into three large clades, indicated that although some patterns were general to all groups, several patterns were lineage-specific. Whether these lineage-specific patterns occurred because of chance, or were due to a higher percentage of certain trait states (e.g. inconspicuous vs. showy flowers) in one group over another remains to be investigated. However, the fact that historical patterns can be interpreted using large-scale phylogenies of extant taxa is encouraging. Future work aimed at untangling the complex web of trait correlations with dioecy in terms of their presence, cause, and priority will be critical for understanding the ecological mechanisms responsible for the evolution of separate sexes from combined sexes in flowering plants.

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Appendix 1 Transition rates for correlations between dioecy and seven ecological traits. For example, rates for clades in which the transition to dry fruits (the negatively correlated trait) upon the background of dioecy is given by q_{43}, while q_{21} is the rate at which dry fruit arises in nondioecious lineages (see Fig. 2). R = Rosids, A = Asterids, and E = Eumagnoliids.

Evolving		Correlated trait		Dioecy		Cosexuality		Negatively correlated	
Background		Sexual system		Trait *Y*		Trait *Y*		Trait sexual system	
Y		q_{12}	q_{34}	q_{13}	q_{24}	q_{42}	q_{31}	q_{43}	q_{21}
Fruit	R	0.08	0.85	0.05	0.002	0.24	0.40	0.77	0.18
	A	0.02	0.001	0.004	0.04	0.17	0.13	0.03	0.06
	E	0.006	2.49	0.01	0.00	0.12	0.01	0.64	0.02
Distribution	R	0.18	1.06	0.07	0.00	1.25	0.06	1.36	0.62
	A	0.34	0.55	0.00	0.16	1.29	0.00	0.09	0.15
	E	0.01	1.22	0.01	0.00	0.02	0.06	0.17	0.00
Habit	R	0.02	0.01	0.01	0.13	1.94	0.07	0.00	0.06
	A	0.15	1.69	0.00	0.15	0.75	0.00	0.08	0.17
	E	0.03	0.13	0.16	0.07	0.22	2.49	0.00	0.00
Flower	R	0.06	1.22	0.001	0.04	0.18	3.07	0.001	0.06
	A	0.04	0.45	0.01	0.06	0.30	0.00	0.08	0.12
	E	0.03	2.74	0.002	0.11	0.33	0.01	0.16	0.06
Inflorescence	R	0.42	0.00	0.10	0.004	0.014	0.35	0.00	0.18
	A	0.01	2.75	0.01	0.05	0.01	0.35	1.47	0.002
	E	0.004	2.49	0.004	0.01	0.03	0.13	0.70	0.002
Flowers per inflorescence	R	0.04	1.45	0.001	0.03	0.18	0.002	0.54	0.06
	A	0.24	0.00	0.11	0.05	0.18	0.98	0.50	0.23
	E	0.03	0.06	0.01	0.06	0.39	0.06	0.03	0.05
Pollination	R	0.002	0.08	0.01	0.04	0.01	0.20	0.08	0.0003
	A	0.08	0.0001	0.04	2.78	0.002	0.88	1.41	3.20
	E	0.003	0.0008	0.02	0.70	1.89	0.18	0.0003	0.02