

Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera

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Abstract

Darwin recognized that biological diversity has accumulated as a result of both adaptive and nonadaptive processes. Very few studies, however, have addressed explicitly the contribution of nonadaptive processes to evolutionary diversification, and no general procedures have been established for distinguishing between adaptive and nonadaptive processes as sources of trait diversity. I use the diversification of flower colour as a model system for attempting to identify adaptive and nonadaptive causes of trait diversification. It is widely accepted that variation in flower colour reflects direct, adaptive response to divergent selective pressures generated by different pollinators. However, diversification of flower colour may also result from the effects of nonadaptive, pleiotropic relationships with vegetative traits. Floral pigments that have pleiotropic relationships to vegetative pigments may evolve and diversify in at least two nonadaptive ways. (1) Indirect response to selection on the pleiotropically related nonfloral traits may occur (indirect selection). (2) Divergent evolution in response to parallel selective pressures (e.g. selection by pollinators for visually obvious flowers) may occur because populations are at different genetic starting points, and each population follows its own genetic 'line of least resistance.' A survey of literature suggests that pleiotropic relationships between flower colour and vegetative traits are common. Phylogenetically informed analyses of comparative data from *Dalechampia* (Euphorbiaceae) and *Acer* (Aceraceae), based on trait-transition probabilities and maximum likelihood, indicated that floral and vegetative pigments are probably pleiotropically related in these genera, and this relationship better explains the diversification of floral colour than does direct selection by pollinators. In *Dalechampia* pink/purple floral bract colour may have originated by indirect response to selection on stem and leaf pigments. In *Acer* selection by pollinators for visually obvious flowers may have led to the evolution of red or purple flowers in lineages synthesizing and deploying red anthocyanins in leaves, and pale-green or yellow flowers in species not deploying red anthocyanins in vegetative structures. This study illustrates the broader potential of indirect selection and parallel selection on different genetic starting points to contribute to biological diversity, and the value of testing directly for the operation of these nonadaptive diversifying processes.

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Introduction

Darwin (1859, 1877) recognized that diversification of organisms and traits can come about through both adaptive and nonadaptive processes. However, Darwin and evolutionary biologists since have stressed the importance of divergent selective pressures in speciation and trait divergence (herein collectively termed 'adaptive diversification') rather than other, 'nonadaptive' processes that might cause trait divergence (herein termed 'nonadaptive diversification'). This is understandable because adaptation is probably the major source of biological diversity seen today (but see cautionary note in Gould & Lewontin, 1979). Indeed, discounting 'scientific' creationism, almost no modern studies have explicitly considered the possible processes involved in nonadaptive diversification of traits, and no general procedures have been established for distinguishing between adaptive and nonadaptive sources of trait diversity. In the present study I use the diversification of flower colour in two plant genera as a study system to illustrate how one can attempt to identify nonadaptive sources of trait diversity. Although the approach cannot be considered conclusive (because one can never completely exclude undetected adaptation), it provides a useful and parsimonious framework for hypothesis generation and further testing.

The diversity and beauty of flower colour have been appreciated by humans for perhaps as long as our species has existed. Since Darwin's time, variation in flower colour has usually been attributed to direct effects of natural selection generated by pollinator behaviour (e.g. animal colour preferences and floral constancy; Grant & Grant, 1965; Stebbins, 1974; Proctor *et al.*, 1996). Despite alternative explanations posed by Darwin himself (Darwin, 1859, 1877), other causes of evolutionary diversification in floral colours (or any other trait) have remained largely unevaluated (see discussion in Gould & Lewontin, 1979). The availability of modern phylogenetic and evolutionary-genetic information now makes possible more balanced evaluation of the causes of flower-colour diversity, and trait diversification in general. In this paper I suggest that variation in floral colours may also commonly arise through (1) indirect response to selection acting on genetically correlated traits ('indirect selection'; Falconer, 1989), and (2) different evolutionary responses to the same selective pressure acting in parallel on populations at different genetic starting points ('parallel selection in different genetic contexts'). Pleiotropy, the effect of a locus (or several loci) on two or more phenotypic traits, plays a central role in both scenarios.

It is well known that flower colour and pigment chemistry can influence choices made by flower-visiting animals (e.g. Grant, 1966; Kevan, 1983; Waser, 1983; Lunau *et al.*, 1996; Wilbert *et al.*, 1997; Schemske & Bradshaw, 1999) and that pollinator choice can influence plant reproductive fitness (Faegri & van der Pijl, 1979;

Waser & Price, 1981, 1983; Waser, 1983). Together these observations have contributed to the widely held belief that the colour of flowers and the type of pollinator are closely interrelated (e.g. the colour component of 'pollination syndromes'; Stebbins, 1974; Faegri & van der Pijl, 1979; Wyatt, 1983; Proctor *et al.*, 1996; Armbruster *et al.*, 2000; Ollerton & Watts, 2000). However, many questions have been raised recently about the inference of such a simple relationship (Waser, 1983, 1998; Herrera, 1996; Waser *et al.*, 1996; Chittka & Waser, 1997; Ollerton, 1996, 1998; reviewed in Armbruster *et al.*, 2000). First, colour-based choices made by animals are more often the result of associative learning rather than innate preference or visual abilities (Waser, 1983; Chittka & Waser, 1997; Melendez-Ackerman *et al.*, 1997). Also, evolution of flower colour may be more often associated with enhancing pollinator constancy (Grant, 1950; Waser, 1986) than specialization on animals with specific preferences (Waser, 1983; Melendez-Ackerman *et al.*, 1997). Finally, spatial and temporal variation in intensity and direction of pollinator-generated selection may limit plant response to pollinator preferences (Herrera, 1996; Brody, 1997). To my knowledge, the only nonadaptive mechanisms of flower-colour diversification that have been proposed are mutation (e.g. Epperson & Clegg, 1987a) and genetic drift (e.g. Epling & Dobzhansky, 1942; Wright, 1943; Wilson & Thomson, 1996), although these are not considered major contributors to diversity in the absence of selection (but see discussions of other nonadaptive mechanisms in Armbruster, 1996; Wilson & Thomson, 1996; Levin, 2000). The purpose of this study is to evaluate two additional possible mechanisms of flower-colour diversification (where trait diversification is defined as an evolutionary increase in the number of character states in a lineage): indirect selection and parallel selection in different genetic contexts. I do this by deriving from each mechanism macroevolutionary predictions that can be tested with phylogenetic information via a relatively new method based on character-state transition probabilities.

Indirect selection, genetic context and their macroevolutionary predictions

It has been recognized recently that floral traits are sometimes genetically correlated with other floral (e.g. Schwaegerle & Levin, 1991; Conner & Via, 1993; Campbell *et al.*, 1994; Conner & Sterling, 1995; Campbell, 1996) or vegetative traits (Schwaegerle & Levin, 1991; Conner & Sterling, 1996; Armbruster *et al.*, 1999). This leads to the possibility that floral traits may be genetically correlated with other traits that directly affect fitness, and floral traits may sometimes respond to indirect selection (Lande & Arnold, 1983; Falconer, 1989; Campbell *et al.*, 1994; Melendez-Ackerman *et al.*, 1997). As the correlated floral trait is not the target of selection, its evolution

can be considered 'nonadaptive' (or sometimes even maladaptive, in the case of multi-trait stabilizing selection). Although these studies considered floral morphological traits, there could also be pleiotropic relationships between flower colour and other phenotypic traits. For example, Rausher & Fry (1993), Fineblum & Rausher (1997) and Simms & Bucher (1996) have pointed out potential biosynthetic and other pleiotropic relationships between floral pigments and compounds that deter herbivores on *Ipomoea purpurea* (Convolvulaceae). Changes in the production of one group of compounds may affect the production of other compounds. Thus flower colour may be influenced by pleiotropic relationships with various other floral or nonfloral traits, and flower colour may evolve by indirect response to selection on those traits (Levin & Brack, 1995; Armbruster, 1996; Fineblum & Rausher, 1997; Melendez-Ackerman *et al.*, 1997; Simms & Bucher, 1997), and this may lead to 'nonadaptive radiation' of the trait (Levin, 2000, p. 37). Recent research has greatly strengthened and expanded the list of possible phytoprotective functions of vegetative anthocyanins (Graham, 1998; Chalker-Scott, 2001; Gould *et al.*, 2001; Hamilton & Brown, 2001; Holbrook *et al.*, 2001; Starr & Oberbauer, 2001; Zufall & Rausher, 2001), and hence the evolution of flower colour by indirect response to selection on vegetative anthocyanins seems at least possible. Evolution of flower colour by indirect selection through another trait should lead to a tight association, across species, between flower colour and the other trait. In a study in which the history of trait changes can be traced on the group's phylogeny, divergence as a result of indirect selection should cause both traits to change simultaneously (i.e. on the same branches) on the phylogeny (Fig. 1a; see also Martins, 2000). The same macroevolutionary pattern can also be generated by correlational selection (*sensu lato*, including additive and interactive effects of multiple traits on fitness; Endler, 1986, 1995; Armbruster & Schwaegerle, 1996; cf. Herrera, 2001), as is discussed more thoroughly below.

A second mode of nonadaptive diversification of traits is by a single selection pressure operating in parallel on

two or more populations at different genetic starting points (genetic contexts). The genetic context is the web of interacting pleiotropic and linkage relationships among traits, as well as the presence or absence of 'critical' alleles in the population. Darwin (1859, 1877) and others since, recognized that parallel selective pressures operating on different starting points may lead to a diversity of adaptive responses, with trait diversity being the result of chance preconditions rather than divergent selection (Stebbins, 1950, 1974). Indeed, exaptation (or pre-adaptation; Gould & Vrba, 1982) sometimes occurs in this manner, with a stochastic pre-adaptation being responsible for an evolutionary transition and hence diversification. Thus among-population and among-species variation in flower colour may sometimes arise by evolutionary response to a single selective pressure operating in parallel on different populations. An example might be selection by pollinators for visually obvious (showy) flowers, i.e. spectral combinations that differ from green background, regardless of the exact colour (see Spaethe *et al.*, 2001). Given different genetic starting points in different populations, divergence may occur as each population follows its own genetic 'line of least resistance' although evolving in response to the same selective pressure (Stebbins, 1950, 1974; Armbruster, 1996; Schluter, 1996). For example, populations already synthesizing anthocyanins for protection of vegetative structures would be more likely to evolve anthocyanin-pigmented flowers in response to pollinator selection for showiness than would populations lacking anthocyanin biosynthesis. This process should generate an association between flower colour and the conditioning trait (e.g. presence of vegetative anthocyanins) across species. In a study in which the history of trait changes can be traced on the group's phylogeny, divergence in flower colour as a result of genetic context should be reflected in changes in the contextual trait(s) (e.g. origin of anthocyanin synthesis) occurring prior (on each relevant branch) to changes in flower colour (Fig. 1b; see also Donoghue, 1989).

Natural selection will usually operate on floral and vegetative traits somewhat independently, as their

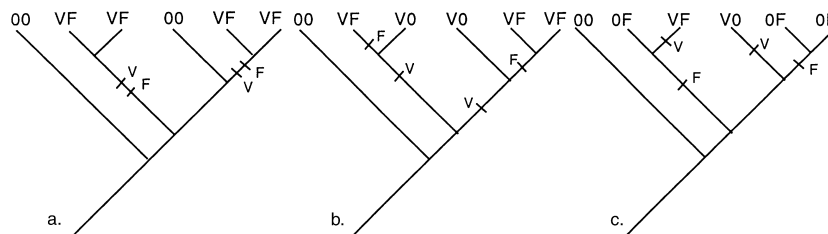


Fig. 1 Three phylogenetic scenarios depicting the expected patterns of trait transition and distribution under: (a) indirect selection as a result of pleiotropic relationship between expression of floral anthocyanins (F) and vegetative anthocyanins (V), or strong correlational selection; (b) parallel selection in multiple genetic contexts with the state of V influencing the evolution of F, or correlational selection, with V influencing F; (c) independent evolution of F and V in response to direct selection on each.

functions are so different (Berg, 1960), and thus if the traits were genetically independent they would usually show no relationship on the phylogeny (Fig. 1c). Sometimes, however, natural selection operates in a correlated fashion on two genetically independent traits (Endler, 1995; Armbruster & Schwaegerle, 1996), and this can create the same patterns on a phylogeny as indirect selection or parallel selection in diverse genetic contexts (Fig. 1a,b; Donoghue, 1989; Armbruster, 1992). Thus distinguishing trait diversity that is the product of correlational selection (selective covariance) from that produced by indirect or parallel selection depends on other sources of data, such as analysis of functional relationships between traits (e.g. Armbruster, 1990; Conner & Via, 1993; Conner & Sterling, 1995; Armbruster & Schwaegerle, 1996; Herrera, 2001). One form of correlational selection that is especially problematic in the present context is when pigments in leaves and flowers have similar protective functions (e.g. against photo-damage, drought-stress, chilling, herbivores, etc.). An environment selecting for pigmented stems would also select for pigmented flowers and individuals with pigments deployed in both organs might have highest fitness (Armbruster & Schwaegerle, 1996). In this case the former may act as a pre-adaptation (pre-adaptation) for the latter, or vice versa (see Armbruster, 1997). The macroevolutionary pattern generated by this relationship would be very similar to that generated by indirect or parallel selection, hence requiring other sources of data to sort the causes out (see below).

The roles of indirect selection and parallel selection in multiple genetic contexts as sources of variation in floral colours has received very little attention. I will address the issue by (1) reviewing recent literature on floral pleiotropy to assess how commonly flower colour is pleiotropically related to other traits, and (2) presenting results of phylogenetically based comparative analyses of flower colour, pollination and vegetative pigments in two representative, unrelated plant genera, *Dalechampia* vines (Euphorbiaceae) and maple trees (*Acer*; Aceraceae).

Flower-colour pleiotropy

How common are pleiotropic relationships between flower pigments and vegetative traits? To address this question I searched the recent literature in the ISI database (1991–98; Institute for Scientific Information, Inc., 1998), and references cited therein. I was surprised by how many articles I found, especially on cultivated plants, that documented pleiotropic relationships between flower colour and vegetative traits.

Schoen *et al.* (1984) reported flower and stem colours being pleiotropically related in *Ipomoea purpurea* (Convolvulaceae), and Epperson & Clegg (1988, 1992) elaborated on the genetics of this correlation. Rausher & Fry (1993) noted that flower colour is pleiotropically related to plant vigour in the same species. Rausher & Fry

(1993), Fineblum & Rausher (1997), and Simms & Bucher (1997) have also pointed out potential pleiotropic and biosynthetic relationships between floral pigments and compounds that deter herbivores on *I. purpurea*, and that changes in the production of one group of compounds may affect the production of other compounds. Studies on *Clarkia* (Onagraceae; Bowman, 1987) and *Phlox* (Polemoniaceae; Levin & Brack, 1995) also document pleiotropic links between floral colour and vegetative colour or vigour. Similar results are reported for cultivated maize (Poaceae; Coe *et al.*, 1988). I can add to these observations by noting an apparent pleiotropic relationship in *Syringa vulgaris* (lilac) cultivars: those with lilac flowers have darker, apparently anthocyanin-rich, more frost-resistant leaves than cultivars with white flowers. Similarly in potatoes (*Solanum tuberosum*), cultivars in Norway with red-skinned tubers generally have purplish flowers, and those with yellow/brown-skinned tubers generally have white flowers (Armbruster, unpublished data).

Detailed genetic studies, especially of agricultural species, have greatly enhanced our understanding of pleiotropic influences on flower colour. Studies of the genetics of sepal colour in *Melilotus alba* have shown that a single locus influences both sepal colour and stem and leaf colour (Goplen, 1992). Flower colour and seed-coat colour have been found to be pleiotropically associated or genetically linked in *Phaseolus vulgaris* (Bassett, 1995). Genetic research in soyabeans (*Glycine*) has also shown that a single locus controls the expression of anthocyanin pigments in both flowers and the hypocotyledonous (lower) stem (Yu *et al.*, 1994).

In the case of potatoes noted above, the genetic basis of this pleiotropic relationship has been worked out in some detail by Vaneck *et al.* (1993, 1994). The D locus, on chromosome 2, controls the synthesis of red anthocyanins throughout the plant body, hence in both flowers and tubers. Locus P, on chromosome 11, controls the production of blue anthocyanins. The F locus, on chromosome 10, is involved in flower-specific expression of the anthocyanin genes, as accommodated by the D and P loci. An additional one or two loci are involved in tuber-specific expression of the anthocyanin genes. Thus the D and P loci create the pleiotropic relationship (and phenotypic correlation) between flower and tuber colour, whereas the other loci tend to weaken the association between the two traits. Because the genetics and biosynthesis of anthocyanins are thought to be highly conserved across angiosperm groups (e.g. Graham, 1998), it seems reasonable to use this genetic system in potatoes as a working model for other dicot species.

In summary, it appears that pleiotropic connections between flower colour and vegetative traits are relatively common, and thus such pleiotropic relationships may sometimes influence the evolution of flower colour. This pleiotropic influence on flower-colour evolution may involve indirect selection (Levin & Brack, 1995;

Fineblum & Rausher, 1997; Melendez-Ackerman *et al.*, 1997; Levin, 2000; Martins, 2000; S. Y. Strauss, pers. comm.) or parallel selection in multiple genetic contexts (Armbruster, 1996).

Comparative analyses: materials and methods

New methods for analysing the evolution of discrete traits, based on applying continuous-time, Markov transition probabilities in a phylogenetic context, and maximum-likelihood estimation (e.g. DISCRETE; Pagel, 1994, 1997, 1999; Brook Milligan, pers. comm.), offer new opportunities for estimating historical order of appearance of traits and assessing functional and pleiotropic relationships among them. This approach uses probabilities of transition between character-state combinations [e.g. for two binary characters: (0, 0) to (0, 1); (0, 0) to (1, 0); (0, 1) to (1, 1), etc.] in a tree-wide maximum-likelihood framework (see Fig. 2), and thereby avoids the problem of estimating ancestral conditions. Given a resolved phylogeny, we should be able to detect pleiotropy as an overall (phylogenetically corrected) correlation between two traits (Pagel's 'omnibus' test, which uses Monte Carlo randomization to estimate P -values), indirect selection as simultaneous change in both traits (Fig. 1a), and diversification through effects of different genetic contexts as ordered appearance of traits (Fig. 1b), assessed by tests for temporal order of trait change and trait contingency, with results compared with the χ^2 distribution (see Table 1 and Fig. 2). Note that in this model, the transition (00 \rightarrow 11) expected by the indirect selection hypothesis, is actually assumed to be impos-

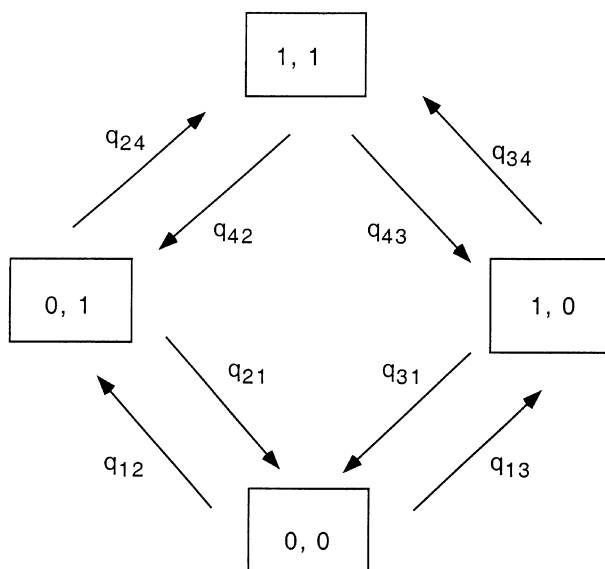


Fig. 2 Possible character transitions assumed in the Markov model used to analyse the comparative data employing DISCRETE (Pagel, 1994, 1999). q 's are estimated transition probabilities.

ible; this expectation is instead reflected by the following inequality of probabilities (P): [$P(00 \rightarrow 01) + P(00 \rightarrow 10)$] \ll [$P(01 \rightarrow 11) + P(10 \rightarrow 11)$] (Pagel, 1994).

To test the idea that flower-colour evolution may have been influenced by pleiotropic relationships with other traits, I examined the relationship between inferred evolutionary changes in flower colour and related pigments in vegetative structures. I examined this relationship in two unrelated plant genera, *Dalechampia* (Euphorbiaceae) and *Acer* (Aceraceae/Sapindaceae) using molecular phylogenetic information to estimate the history of character evolution.

All analyses of colour described below were based on colours perceived by humans rather than the pollinating animals. This introduces error and may increase the type-II error rate (failing to detect a relationship when it exists). However, it seems unlikely to affect the type-I error rate (finding a relationship when none exists). The error introduced by this simplification can be described as follows. Some portion of floral-colour variation that is perceived by pollinators is undetected by us. This leads to misclassification, but it should usually be random (unbiased) with respect to our null and alternative hypotheses. Another source of error is variation in flower colour that is detected by us, but not by the pollinators. This also leads to misclassification, but again it should usually be independent of our null and alternative hypotheses, and also introduces random noise in the analysis. Both sources of error are probably quite small, because spectral sensitivities of both insects and humans are quite good, despite their differences. Nevertheless, statistical noise may be increased and the statistical signal-to-noise ratio may be depressed. This may increase slightly our failure to detect relationships that actually exist, but should not affect the likelihood of concluding there is a relationship when there is none.

Philosophical underpinnings of the analysis

Darwin (1877) and many since have noted that most traits that appear to be nonadaptive may actually have unknown adaptive significance. However, if we are to undertake the scientific study of adaptive processes, we must be able to falsify adaptive hypotheses (Gould & Lewontin, 1979). Acceptance of adaptation as the null condition is preferred by some (see Eberhard & Gutierrez, 1991), but it makes study of adaptation difficult. An arguably better approach is to treat nonadaptive explanations as more parsimonious than adaptive explanations, and to accept adaptive explanations only after rejecting simpler hypotheses as inadequate. Thus here I treat pleiotropy as a simpler explanation of trait covariance than correlational selection, for example (see Armbruster & Schwaegerle, 1996), and hence place the burden of proof on demonstrating that adaptation alone can explain a particular pattern. This does not mean that

Table 1 Alternative hypotheses, predictions and statistical tests for correlated evolution of flower colour and vegetative pigments. Note that results of parallel evolutionary response by flowers and vegetative structures to the same selective pressure (a form of correlational selection) cannot be distinguished from those of pleiotropy (although considered here to be less parsimonious), and must be tested in other ways.

Hypothesis	Prediction	DISCRETE test statistic
H ₀ : No pleiotropic relationship between flower and vegetative colour/pigments	Distributions of flower and vegetative colour/pigments on phylogeny are independent	Fail to reject H ₀ of omnibus test
H _{1A} : Indirect selection operated on flower colour (generated by direct selection on vegetative pigments), or H _{1B} : Indirect selection operated on vegetative pigments (generated by direct selection on flower colour)	1. Distributions of flower and vegetative colour/pigments on phylogeny are not independent 2. Changes in floral colour and vegetative traits occur simultaneously on phylogeny	Reject H ₀ of omnibus test Fail to reject H ₀ of temporal-order test $q_{24} + q_{34} \gg q_{12} + q_{13}$
H _{2A} : Genetic context (vegetative pigments) has influenced the evolution of flower colour	1. Distributions of flower and vegetative colour/pigments on phylogeny are not independent 2. Changes in vegetative colour/pigments precede changes in floral colour on phylogeny 3. State of vegetative colour/pigments affects probability of evolutionary change in flower colour	Reject H ₀ of omnibus test Reject H ₀ of temporal-order test; $q_{13} > q_{12}$ Reject H ₀ of contingent-change test; $q_{34} > q_{12}$
H _{2B} : Genetic context (flower colour) has influenced the evolution of vegetative pigments	1. Distributions of flower and vegetative colour/pigments on phylogeny are not independent 2. Changes in floral colour precede changes in vegetative colour/pigments on phylogeny 3. State of flower colour affects probability of evolutionary change in vegetative colour/pigments	Reject H ₀ of omnibus test Reject H ₀ of temporal-order test; $q_{12} > q_{13}$ Reject H ₀ of contingent-change test; $q_{24} > q_{13}$

natural selection is assumed to be less common, only that it is complex and interesting, and deserves to be treated as the alternative hypothesis. Thus the conclusion that a trait or property is not the result of direct natural selection is only provisional because additional data may ultimately reverse the balance of evidence (Darwin, 1859), as is consistent with the role of theory and falsification in the hypothetico-deductive method (Popper, 1963; Mayr, 1982).

***Dalechampia* (Euphorbiaceae)**

Species of *Dalechampia* exhibit large variation in colour of blossom structures, as well as pollination ecology. The blossoms usually comprise two large showy bracts, three pistillate flowers, c. 10 staminate flowers, and in most species a resin-secreting gland, which supplies pollinating bees with a reward: nest-building material. The bracts are technically vegetative structures, but they are modified 'for' floral function and attract pollinators (Armbruster, 1997; Antonsen & Armbruster, unpublished data). Bract

colours include green, white, yellow, pink and purple; this diversity has been attributed to adaptation to different pollinators (Webster & Webster, 1972). My goal was to determine if this diversity is best attributed to response to divergent selective pressures generated by different pollinators, or whether other factors, such as indirect or parallel selection, have played a role.

To test for an adaptive association between pollination system and floral colours, I classified a sample of 38 species of *Dalechampia* for which data were available (representing a broad range of taxonomic diversity in the neotropics, where most species occur) into three pollination modes, four classes of bract colour and three classes of resin colour. I tabulated frequencies of colour and pollinators (Tables 2 and 3) and tested for associations using log-linear analysis (Sokal & Rohlf, 1981; Wilkinson, 1988) of number of evolutionary origins of each association (Ridley, 1983; Felsenstein, 1985; Harvey & Pagel, 1991). I used parsimony as implemented in MacClade 3.1 (Maddison & Maddison, 1992) to reconstruct character evolution on a phylogeny estimated from

Table 2 Colour of involucral bracts vs. pollination mode in a broad, opportunistic (haphazard) sample of neotropical species of *Dalechampia*, representing all sections of the genus. Numbers in columns 2–9 are number of independent origins of the trait association (determined from optimizing character states onto the ITS/*trnK* phylogeny using parsimony, without forward or reverse transformation biases or assumptions about accelerated or delayed transformations), and in parentheses the number of species with the trait association. There was no evidence for a relationship between bract colour and pollination mode (based on number of origins, log-likelihood $\chi^2 = 1.43$, $P = 0.23$), nor for a relationships between pollination mode and presence or absence of anthocyanin pigments in the bract (log-likelihood $\chi^2 = 0.21$, $P = 0.90$).

Pollination mode	Bract colour					Total with anthocyanins	Total without anthocyanins
	Green/ Pale green	White	Pink/ magenta	Yellow	Purplish- brown		
Male euglossines (fragrance collecting)	2 (3)	2 (2)	1 (2)	0 (0)	0 (0)	1 (2)*	4 (5)*
Female euglossines	3 (6)*	6 (11)*	2 (3)	0 (0)	0 (0)	2 (3)*	9 (17)*
Female <i>Hypanthidium</i>	4 (6)*	1 (1)*	1 (1)	1 (1)	1 (1)	2 (2)*	6 (8)*
Total	9 (15)	9 (14)	4 (6)	1 (1)	1 (1)	5 (7)	19 (30)

*Used in statistical analyses; other cells excluded because of small sample size.

Table 3 Association between the colour of resin reward and the pollination mode in a broad, opportunistic (haphazard) sample of neotropical species of *Dalechampia*, representing all sections of the genus. Numbers in columns 2–6 are number of independent origins of the association, and in parentheses, the number of species (see Table 2 for additional details). There was no evidence for an association between resin colour and pollination mode (based on number of origins, log-likelihood $\chi^2 = 0.36$, $P = 0.55$).

Pollination mode	Resin colour				Total
	Clear/ whitish	Yellow/ orange	Blue/ greenish	Maroon	
Female <i>Hypanthidium</i> / worker <i>Trigona</i>	5 (6)*	3 (3)*	1 (1)	0 (0)	9 (10)
Female euglossines	5 (7)*	6 (11)*	2 (2)	1 (1)	14 (21)
Total	10 (13)	9 (14)	3 (3)	1 (1)	23 (31)

*Used in statistical analyses; other cells excluded because of small sample size.

morphological and molecular data using PAUP 3.1 (Armbruster, 1993, 1997; Swofford, 1993; Armbruster & Baldwin, 1998; additional details on the data are presented in Armbruster, 1996). Finally, to avoid introducing errors by estimating ancestral states (as under parsimony), I used a maximum likelihood approach for evaluating character transition probabilities (DISCRETE; Pagel, 1994), a highly resolved molecular tree (based on combined sequences of nuclear ribosomal ITS and chloroplast *trnK* intron), and branch lengths based on parsimony estimates of base-pair substitutions on the above tree (Armbruster & Baldwin, 1998; Baldwin & Armbruster, unpublished data) to test for (1) correlations between pollinators and floral bract colour (omnibus test) and (2) whether pollinators influence the evolution of floral bract colour (trait contingency test).

I also used similar tabular methods, Pagel's (1994) DISCRETE program, and the molecular tree with branch lengths (Armbruster & Baldwin, 1998) to evaluate the

association between floral bract colour and stem colour in 37 species from Old and New World tropics. Stem and bract colours were determined from live plants growing generally in sunny conditions in the field or greenhouse and from photographs taken in the field. The pleiotropy hypothesis predicts an overall correlation between the two traits on the phylogenetic tree (DISCRETE omnibus test). I further predicted that (1) pink/purple bracts are tightly associated with pigmented stems and show simultaneous changes (on the same branches) on the phylogeny (indirect selection hypothesis), or (2) pink/purple bracts evolved more commonly in lineages with anthocyanin-pigmented stems (but after the appearance of anthocyanins) than in lineages with 'unpigmented' (green) stems, thus showing ordered change on phylogeny and contingent relationships among traits (hypothesis of parallel selection in multiple genetic contexts; Table 1, Fig. 1). In the above analyses of species evolution, as well as the ones presented below, I assumed, at least initially, that pleiotropic relationships among traits 'survive' speciation and species divergence intact.

Acer (Aceraceae)

Maples exhibit considerable variation in sepal and petal colour, ranging from red and purple to yellow, green and white. The question I wanted to address was whether this variation has been generated by adaptation to diverse pollinators, by indirect selection or by parallel selection in multiple genetic contexts.

Maples are well known for their bright autumn foliage. Initial observations suggested that the colours of the spring flowers and autumn leaves might be related. This prompted the hypotheses that (1) a single set of genes may affect both flower and leaf pigments (pleiotropy), and either (2) interspecific variation in flower colour is the result of indirect response to selection for protective pigments in the leaves, or (3) interspecific variation in flower colour is the result of parallel selection for

showiness operating in multiple genetic contexts. All three hypotheses lead to the prediction of a statistical correlation between flower and leaf colour across species. The indirect-selection hypothesis leads to the prediction that red and purple flowers appear on the phylogeny simultaneous with anthocyanin-bearing leaves. The genetic-context hypothesis leads to the prediction that red and purple flowers are evolutionarily derived and appear on the same phylogenetic branches as, but later than, leaf anthocyanins, and that the evolution of red/purple flowers is more likely in lineages already synthesizing anthocyanins in the leaves.

I tallied the reported flower colour and autumn leaf colour across the 79 species for which data were available (of the *c.* 124 *Acer* species worldwide; Brockman, 1968; Clapham *et al.*, 1968; Preston, 1976; Edlin, 1978; Harlow *et al.*, 1979; Gleason & Cronquist, 1991; van Gelderen *et al.*, 1994). Subspecies and varieties were treated as distinct only when they differed from the nominate form in flower or leaf colour. I also compared the distribution of flower colours with data on anthocyanin presence (Delendick, 1990) or absence (Ji *et al.*, 1992) in autumn leaves of 106 species and subspecies. I used presence of anthocyanins in autumn rather than spring leaves because the data set was larger. However, the two distributions were highly correlated across species (Delendick, 1990; Ji *et al.*, 1992). This had the advantage over using colour of comparing the actual pigments in the leaves, but it introduced the risk of including irrelevant compounds (anthocyanin-like compounds not creating red or purple colour). Also, the standard methods of anthocyanin extraction and detection are known to yield commonly false positives (Gamon & Surfus, 1999; J. A. Gamon, pers. comm.). I conducted log-linear analysis (using SYSTAT; Wilkinson, 1988) of the species-level association between sepal, petal or stamen colour and autumn leaf colour and pigments.

To conduct phylogenetically informed comparative analyses, I used the *Acer* phylogeny generously provided by David Ackerly (pers. comm.; Ackerly & Donoghue, 1998) based on the sequence of the ITS region of the nuclear ribosomal DNA. This included 37 operational taxa plus the outgroup (*Dipteronia sinensis*), for which I could obtain colour data on 36 taxa. This analysis yielded two equally parsimonious trees. I used Pagel's (1994) program (DISCRETE) to evaluate the association between flower colour and leaf pigments and leaf colour. I employed both trees in these analyses, but the results were virtually identical, and I therefore report the results from only one of the trees. I also tried dropping known wind-pollinated taxa from the analysis, but the results were nearly identical to the previous analysis, and this permutation is not reported here.

The hypothesis of a pleiotropic relationship between flower and leaf colour predicts a positive association between the two (omnibus test). The two additional, mutually exclusive hypotheses predict that (1) red and

purple flowers are tightly associated with red autumn leaves and evolved simultaneously (indirect-selection hypothesis), or (2) red and purple flowers evolved more commonly in lineages with red autumn leaves than in lineages with yellow or dull autumn leaves, and flower colour evolved after leaf colour (genetic-context hypothesis; Table 1).

I repeated the above DISCRETE analyses to compare floral colour with presence of anthocyanins in the autumn leaves, as reported in Delendick (1990) and Ji *et al.*, (1992) (data were combined).

Comparative analyses: results

Dalechampia

Floral-bract colour in *Dalechampia* varies from green to white, pink, purple, or yellow in human vision, exhibiting an apparent adaptive radiation, with frequent parallelisms (Fig. 3). Bracts range from slightly reflective (in some pale green-bracted species) to strongly reflective (in some white- or pink-bracted species) in the ultraviolet wavelengths (Armbruster, unpublished data). Resin colours include clear, whitish, yellow, orange, maroon, blue or greenish in human vision. Resin glands (and the homologous fragrance gland in *D. spathulata* and *D. magnoliifolia*) are generally UV-absorptive. The stigmas and staminate sepals are usually green, but they are sometimes yellow or reddish. Pollination in the neotropics is mostly by female, resin-collecting bees, primarily by the closely related euglossine bees, *Eulaema* spp., *Euglossa* spp. and *Eufriesea* spp. (Apidae; large-glanded *Dalechampia*), or by a few species of *Hypanthidium* (Megachilidae; small-glanded *Dalechampia*). A few closely related species of fragrance-collecting, male euglossine bees (*Eulaema*, *Euglossa*), or pollen-collecting, worker *Trigona* (Apidae) pollinate some neotropical species (Armbruster, 1984, 1988, 1990, 1993). Pollination in Africa is by resin-collecting *Heriades* or *Pachyanthidium* (Megachilidae; Armbruster & Steiner, 1992), and in Madagascar by pollen-feeding beetles and pollen-collecting bees (Armbruster *et al.*, 1993; Armbruster & Baldwin, 1998). Only neotropical species were used in the analysis of pollination presented below, although the floral-vegetative pigment comparisons were based on a broader sample including both neo- and palaeotropical species.

The phylogenetically corrected tabular analyses of the relationship between pollination ecology and floral colours in the neotropics showed no detectable association between euglossine vs. megachilid bee pollinators and floral bract colour (log-likelihood $\chi^2 = 1.43$, $P = 0.23$; Table 2). The DISCRETE analyses also showed no evidence for an association between pollinator and floral bract colour. Comparing bract colour (green/white vs. pink/purple) with pollination (female euglossine bees vs. megachilid bees) yielded a nonsignificant omnibus-test result [DISCRETE omnibus test: likelihood ratio

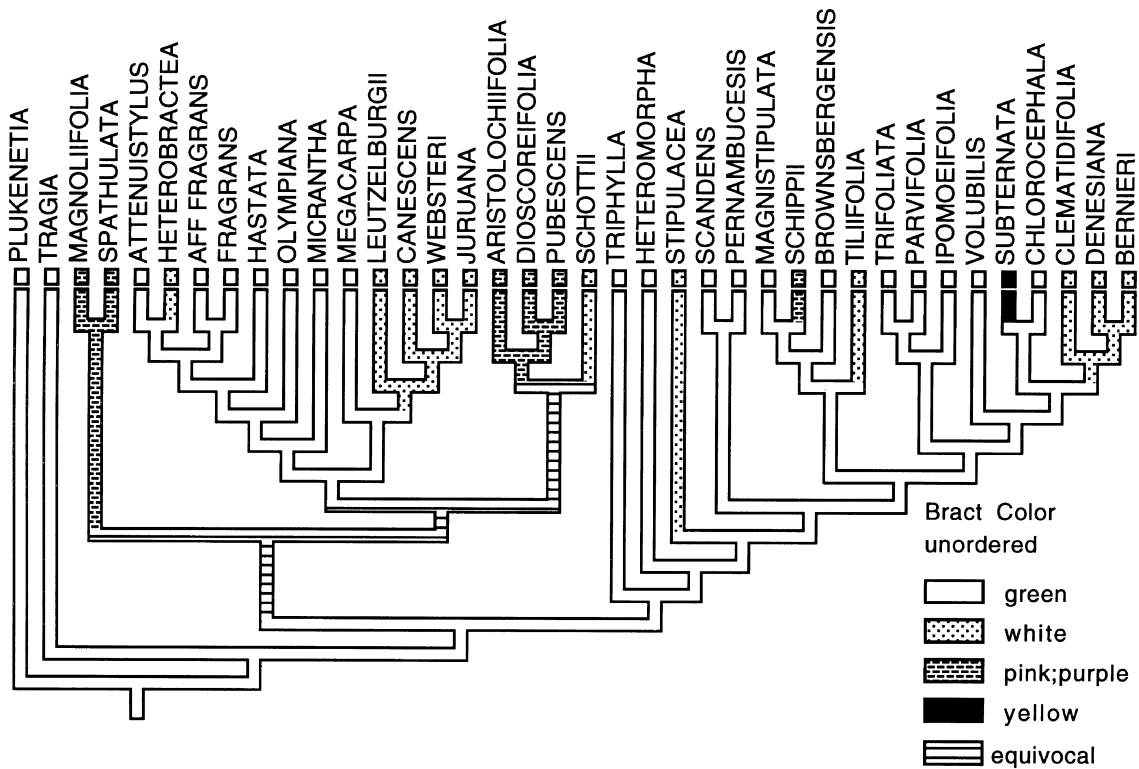


Fig. 3 Estimated phylogeny of neotropical and palaeotropical species of *Dalechampia*, showing a radiation in blossom bract colour. The phylogenetic tree is based on combined nuclear ribosomal ITS and chloroplast *trnK* intron sequences with trait optimization following parsimony without accelerated- or delayed-transformation or forward- or reverse-transformation biases (see Armbruster & Baldwin, 1998).

(LR) = 1.35, $P = 0.30$, calculated from 500 randomizations]. Repeating the analysis with a different classification of pollinator types (female resin-collecting bees vs. male fragrance-collecting bees) also yielded a nonsignificant omnibus-test result (LR = 2.91, $P = 0.17$, calculated from 1000 randomizations). More apropos our interest in anthocyanin evolution, it is clear that the origins of bracts lacking anthocyanins (green, white and yellow) and bracts containing anthocyanins (pink, magenta, purple-brown) are associated in nearly equal proportions with the three main pollinator groups (4 : 9 : 6 and 1 : 2 : 2, respectively; patterns not significantly different from one another: $P = 0.90$; see Table 2). Similarly no evidence for a relationship between pollination ecology and resin colour was detected from tabular analysis of number of origins on the phylogeny (log-likelihood $\chi^2 = 0.36$, $P = 0.55$; Table 3). Together these data suggest little or no relationship between blossom colour and pollinator type in *Dalechampia*, and lead to tentative rejection of the hypothesis that repeated switches between anthocyanin-free and anthocyanin-bearing bracts resulted from selection generated by different types of pollinators. There is also circumstantial evidence against bract and resin colour playing a role in increased constancy, because euglossine bees, at least, readily visit

different-coloured species on the same foraging trips (see Discussion).

In contrast, the predicted association between bract colour and stem and leaf pigments was strongly supported by the distribution of bract colour and stem/leaf colours across species. All species with pink to purple bracts had reddish pigments in their young leaves and stems; species with white or green bracts lacked

Table 4 Association between stem or bud colour and bract colour in a broad, opportunistic (haphazard) sample of neotropical and palaeotropical species of *Dalechampia*, representing all sections of the genus. Numbers in columns 2 and 3 are the number of independent origins of the association, and, in parentheses, the number of species with the trait association (see Table 2 for additional details). The tally of species suggests an association between bract colour and stem/bud colour (log-likelihood $\chi^2 = 27.4$, $P < 0.001$), but the tally of independent origins has too low a sample size for meaningful comparison.

Colour of stem, bud, and/or leaf veins	Bract colour		
	Pink or violet	White or green	Total
Reddish, pinkish, purplish-brown	3–4 (6)	0 (0)	3–4 (6)
Green	0 (0)	1 (31)	1 (31)
Total	3–4 (6)	1 (31)	4–5 (37)

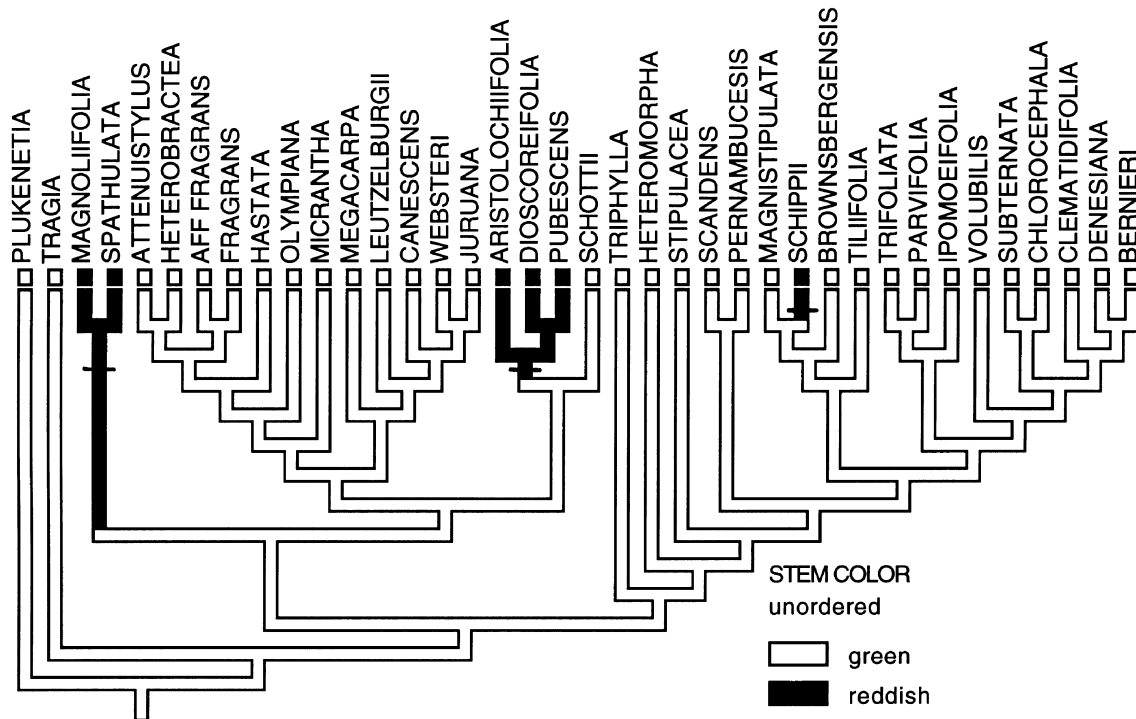


Fig. 4 Phylogenetic distribution of reddish pigments in stems and buds in *Dalechampia*, and inferred origins (slash marks) of pink or purple involucral bracts. Tree and trait optimization is the same as in Fig. 3.

the reddish pigment in leaves and stems (ahistorical association analysis: $P < 0.001$; Table 4); however, because this analysis does not take phylogeny into account, it yields only provisional support for the prediction. Better support is provided by the strong association between the origin of pink and purple bracts and pigmented stems on the reconstructed phylogeny. Although there were only three inferred origins of pink or purple bracts and pigmented stems (Fig. 4), the DISCRETE omnibus test indicated that this association was highly significant (LR = 22.00, $P < 0.001$, determined by 500 randomizations). These observations support the hypothesis of a pleiotropic relationship between leaf and flower pigments, and are consistent with both the indirect and parallel selection hypotheses.

Pigmented stems and pink bracts appear to have originated simultaneously in all three instances (Fig. 4). The DISCRETE test for stem-pigment change preceding bract-colour change, or vice versa, was not significant (LR = 0.122, $P > 0.50$), as predicted by the indirect selection hypothesis but not the genetic-context hypothesis (Table 1). Furthermore, the hypothesis of indirect selection predicts $q_{24} + q_{34} \gg q_{12} + q_{13}$ (Table 1). Indeed this was the case: $8.758 + 1.873 \gg 0.008 + 0.00002$ (Table 6). These results support the hypothesis of diversification of bract colour by indirect selection rather than by response to parallel selection in diverse genetic contexts.

Acer

The open flowers of *Acer* vary from green to yellow, white, red or purple in colour; this variation has arisen through repeated parallelism and reversals (Fig. 5). Flowers of the vast majority of species are apparently pollinated by a variety of insects, especially bees (de Jong, 1976; Matsui, 1991; Renner, 2001; Armbruster, unpublished observations), although wind pollination occurs in *A. negundo* and its relatives, and ambophily (pollination by animals and wind) may occur in many species (C. Herrera, pers. comm.). *Acer platanoides* in Norway, for example, has bright yellow, nectariferous flowers that are highly attractive to *Bombus* spp., *Andrena* spp., *Apis mellifera* (honey bees), and a variety of flies in the earlier spring, and at least the first three groups provide effective pollination (Armbruster, unpublished observations). *Acer pseudoplatanus* in Norway has yellowish-green flowers that are visited and probably pollinated by honey bees and flies in the late spring (Armbruster, unpublished observations). Similar observations of pollination by bumble bees (*Bombus* spp.), honey bees and various small solitary bees have been reported for four nectariferous Japanese species, including both yellow- and red-flowered species (Matsui, 1991). Matsui's (1991) observation of similar pollination of red- and yellow-flowered species suggests that flower colour alone does not alter the pollination ecology of

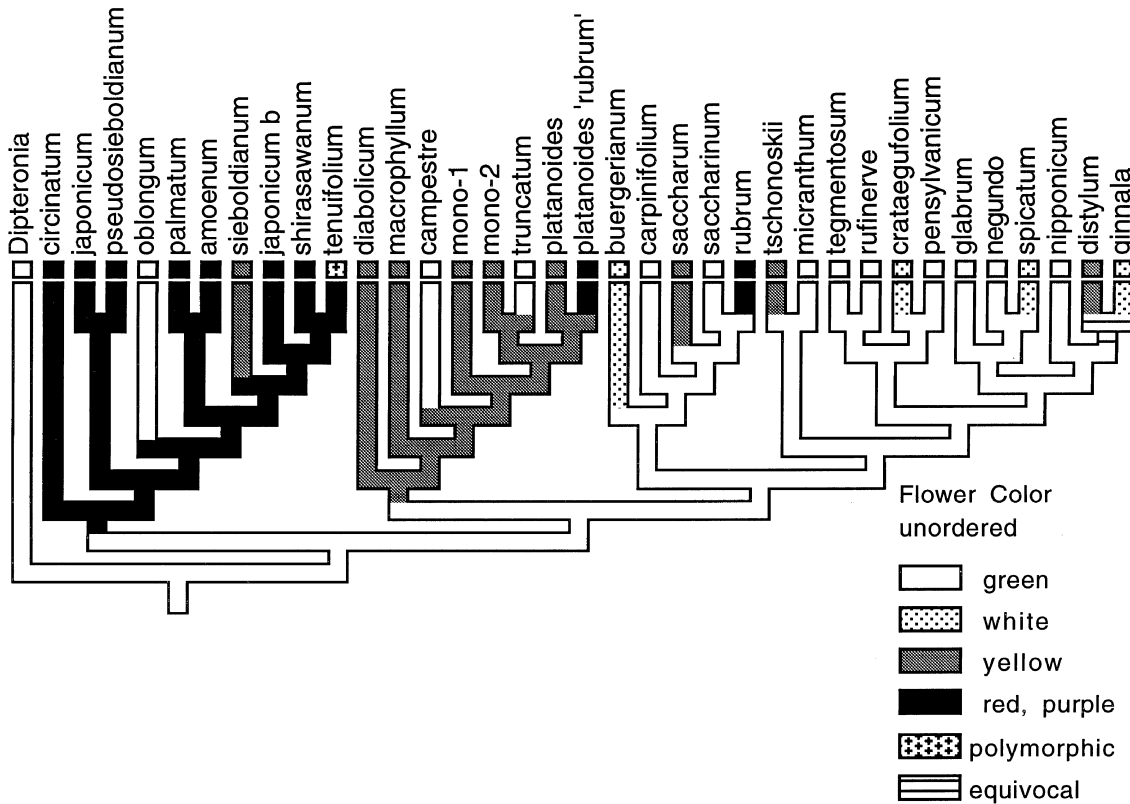


Fig. 5 Estimated phylogeny of *Acer* species, showing diversification in flower colour. Tree is derived from the ITS tree presented in Ackerly & Donoghue (1998). Trait optimization as in Fig. 3.

maples. The wind-pollinated taxa also seem to fit within the same range of colours: for example, the putatively obligate wind-pollinated species in the *A. negundo* complex have greenish flowers. Thus we do not have any evidence for major differences in flower colour being related to pollination: insect-pollinated species have green, yellow or red flowers, and wind-pollinated species have green flowers. The pollination of most maple species remains to be studied in any detail, however, and these conclusions are tentative.

For the present analysis, in the absence of information to the contrary, I will assume that all maples, except for a few wind-pollinated species, have reasonably similar, generalized insect (see Waser *et al.*, 1996) or mixed insect and wind pollination (de Jong, 1976; Renner, 2001). If most *Acer* have generalized insect or mixed insect and wind pollination, then it seems likely that all lineages (outside of the *A. negundo* complex) experience similar selective pressures generated by pollinators.

A simple tally of species suggested that there is an association between flower colour and autumn leaf colour across the sample of *Acer* species studied ($P = 0.002$; Table 5). Generally, species with red petals and/or sepals have reddish autumn foliage, and species with yellow to greenish petals and/or sepals have

yellow or dull brownish autumn foliage. A simple tally of species also suggests an association between red flower colour and the chemical detection of anthocyanins in the autumn leaves ($P = 0.023$; Table 6). Notably,

Table 5 Association between floral colour and autumn leaf colour in an opportunistic sample of species of *Acer* (those for which van Gelderen *et al.* (1994) gave information on flower and leaf colour). Columns 2 and 3 show the number species with the trait association. A tally of species suggests an association between flower colour and autumn leaf colour (but see caution in text about using phylogenetic data; log-likelihood $\chi^2 = 9.8$, $P = 0.002$, using delta correction = 3 for low expected cell values).

Leaf colour	Flower colour		Total
	Yellow/green/white	Red/purple	
Yellow/brown	45*	4*	49
Red (including polymorphic with red)	10*	8*	18
Orange, orange-yellow	9	3	12
Total	64	15	79

*Used in statistical analysis. Orange, orange-yellow were not used in the analyses because this category was ambiguous for presence of anthocyanins and had a small sample size.

Table 6 Association between sepal and/or petal colour and presence of anthocyanins in autumn leaves in a sample of species of *Acer* [those for which van Gelderen *et al.* (1994) gave flower colour and Delendick (1990; pigment presence only) or Ji *et al.* (1992; pigment presence and absence) gave pigment information]. Numbers in columns 2 and 3 are the number species with the trait association. The tally of species suggests an association between flower colour and presence of autumn leaf anthocyanins (but see caution in text about use of phylogenetic data; log-likelihood $\chi^2 = 5.19$, $P = 0.023$).

Leaf anthocyanins	Flower colour		Total
	Yellow/green/ white	Red/ purple	
Absent	16	1	17
Present	39	17	56
Total	55	18	73

of 18 red/purple-flowered taxa with anthocyanin data, 17 had anthocyanins detected in autumn foliage. These observations support the hypothesis of a pleiotropic relationship between leaf and flower pigments and that indirect selection or selection for greater attractiveness has led to red flowers in lineages synthesizing anthocyanins and yellow flowers in lineages not synthesizing anthocyanins. The tendency of red leaf anthocyanins to occur more widely in the phylogeny than red flowers (Figs 5 and 6) supports qualitatively the hypothesis that genetic context rather than indirect selection generated

the diversity of flower colour (compare Fig. 6 with Fig. 4).

The DISCRETE omnibus test indicated that the association between autumn leaf colour and flower colour was significant (LR = 9.340, $P = 0.02$, based on 1000 randomizations), consistent with the existence of a pleiotropic relationship between the two. The analysis of temporal precedence indicated that changes in flower colour occurred after changes in leaf colour ($q_{13} \gg q_{12}$; LR = 6.256, $P = 0.01$, estimated from χ^2 distribution; Tables 1 and 7). Leaf colour appears to have influenced the evolution of flower colour ($q_{34} \gg q_{12}$; Table 7), but the difference was marginally significant (LR = 3.051, $P = 0.08$, estimated from χ^2 distribution).

The DISCRETE omnibus test indicated a marginally significant association between autumn leaf anthocyanins and flower colour (see Fig. 7; LR = 5.915, $P = 0.08$, based on 1000 randomizations). Changes in flower colour appeared to occur after changes in leaf pigments ($q_{13} \gg q_{12}$; Table 7), but the difference in transition probabilities was not significant (LR = 0.2, $P > 0.5$; estimated from χ^2 distribution). Leaf pigments appeared to have influenced the evolution of flower colour, not vice versa ($q_{34} \gg q_{12}$; Table 7), but the difference in transition probabilities was also not significant (LR = 0.1, $P > 0.5$, estimated from χ^2 distribution).

Taken together these results support the hypothesis of a pleiotropic relationship between flower colour and leaf colour/anthocyanins, and the leaf colour data indicate that this relationship has influenced the evolution of

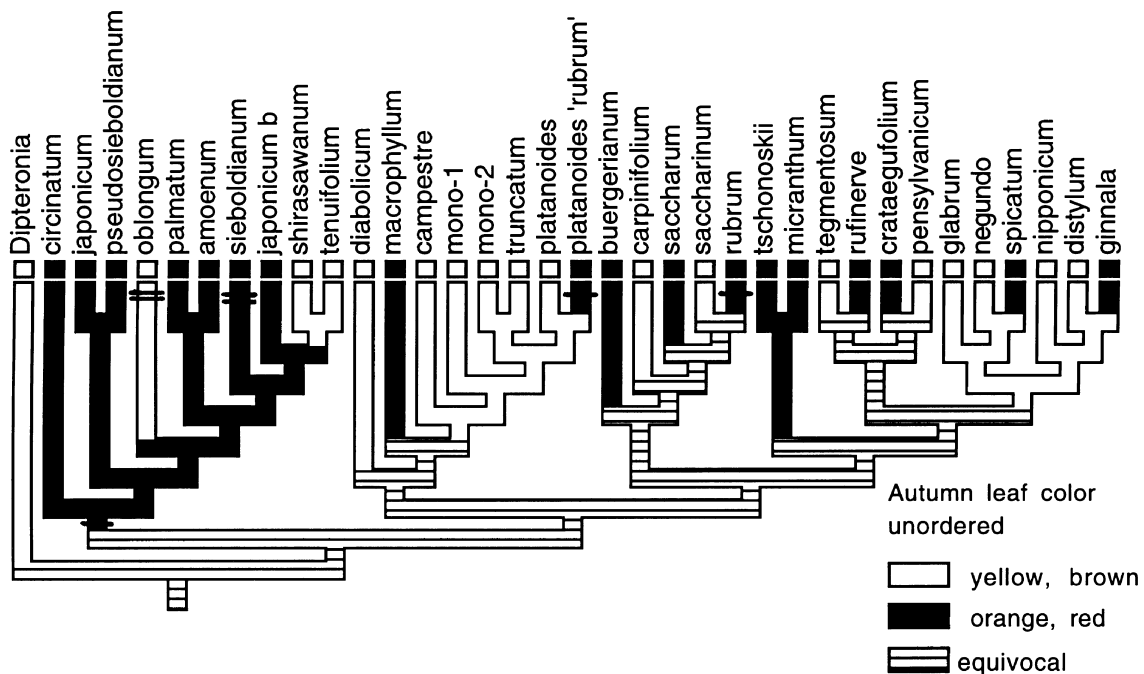
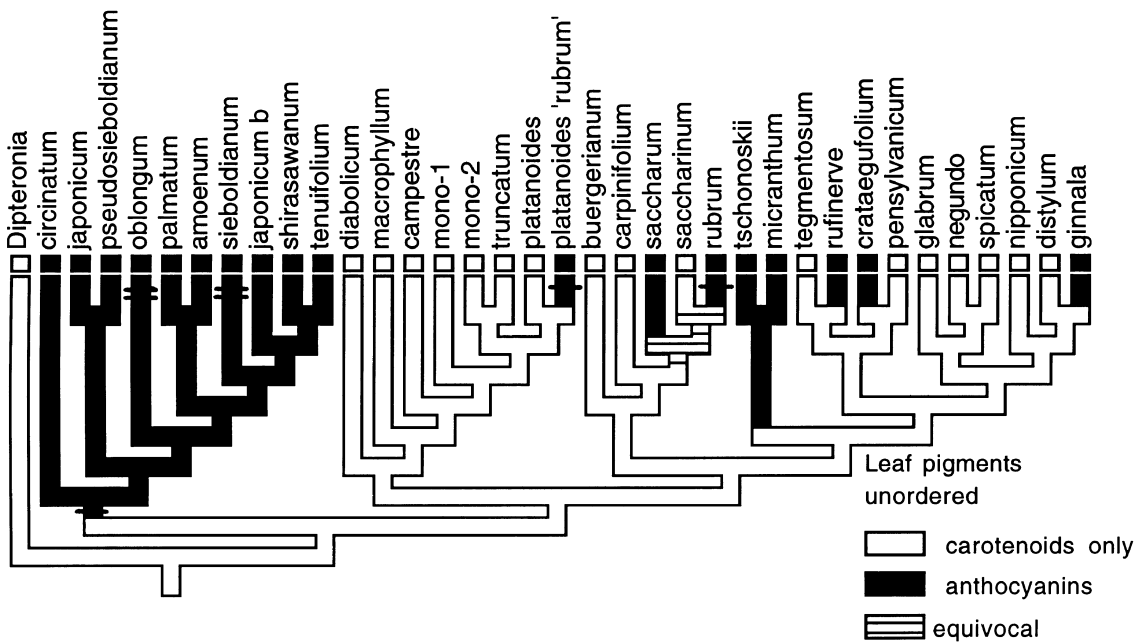


Fig. 6 Phylogenetic distribution of autumn leaf colour in *Acer*, and inferred origins (slash marks) and losses (double slash marks) of red or purple flower parts. Tree is the same as in Fig. 5.

Table 7 Transition probabilities estimated from DISCRETE (Pagel, 1994). See Fig. 2 for explanation of the transition probabilities (q 's).

Analysis	q_{12}	q_{13}	q_{24}	q_{34}	q_{42}	q_{43}	q_{21}	q_{31}
<i>Dalechampia</i>								
Bract colour × euglossines vs. megachilids	8.466	0.086	0.180	0.071	0.075	0.0001	0.400	0.003
Bract colour × male vs. female bees	0.148	0.017	0.0001	0.0176	0.028	0.009	0.603	0.036
Pigmented stems × bract colour	0.008	0.00002	8.758	1.873	0.005	0.051	0.675	2.299
<i>Acer</i>								
ITS tree, leaf colour × flower colour	0.0002	0.706	0.002	0.257	0.072	0.208	0.002	1.329
ITS tree, leaf anthocyanins × flower colour	0.002	0.811	0.474	0.099	0.0002	0.159	1.280	0.280

**Fig. 7** Phylogenetic distribution of anthocyanin pigments in autumn leaves of *Acer*, and inferred origins (slash marks) and losses (double slash marks) of red or purple flower parts. Tree is the same as in Fig. 5.

flower colour. The evidence is consistent with the hypothesis of nonadaptive diversification of flower colour caused by parallel selection in multiple genetic contexts rather than by indirect selection.

Discussion

Is floral-vegetative pleiotropy common?

How widespread are pleiotropic relationships between flower and vegetative traits, and how often might the evolution of one trait affect the evolution of the other? The genetic research reviewed above documents pleiotropic relationships between floral and vegetative pigments or between floral pigments and vegetative traits affecting fitness in many unrelated species of plants. Thus it appears that pleiotropic connections between flower

colour and vegetative traits are relatively common and have the potential to influence flower-colour evolution.

Given the existence of pleiotropic relationships between floral and vegetative pigments, there are at least two distinct ways in which they may contribute to the evolutionary diversification of flower colour. These are: (1) divergent indirect selection and (2) parallel selection acting in diverse genetic contexts. The data presented here suggest that both have operated in flowering plants.

Blossom-colour evolution in *Dalechampia*

There was no discernible relationship between pollination ecology and presence vs. absence of anthocyanin pigments in the blossoms of *Dalechampia*. This observation does not preclude selection on flower colour for

enhanced constancy, but this hypothesis was not supported by field observations of foraging by female euglossine bees. These bees moved indiscriminately between pink-bracted and white-bracted *Dalechampia* species, collecting both maroon and yellow resins, respectively (Armbruster & Herzig, 1984). Additional evidence against this hypothesis comes from analysis of bract colours of sympatric species that may share pollinators. Despite considerable evidence for character displacement and nonrandom community structure in relation to time of bract opening, gland size, and other blossom size and shape variables (Armbruster, 1985, 1986; Hansen *et al.*, 2000), there is no discernible community structure in relation to bract or gland colour (Armbruster, unpublished data).

In contrast, the presence of anthocyanins in blossom structures was significantly associated with their presence in vegetative organs. Together, these observations and analyses suggest that the purplish-reddish pigments (anthocyanins) may have originated in some species of *Dalechampia* for reasons other than attraction of new pollinators. For example, anthocyanins deployed in stems and leaves likely originated as phytoprotectants, protecting against photo-damage, drought stress, or possibly herbivores and pathogens (Graham, 1998; Chalcker-Scott, 2001; Gould *et al.*, 2001; Hamilton & Brown, 2001; Holbrook *et al.*, 2001; Starr & Oberbauer, 2001; Zufall & Rausher, 2001). This shift in vegetative pigments may have led to the simultaneous shift in bract colour (from white or pale green to pink or purple) as a result of the pleiotropic relationship between pigment expression in bracts and stems and leaves (indirect selection).

An alternative explanation for the evolutionary relationship between bract and stem colour cannot be excluded. It is possible that selection by pollinators has generated bract-colour diversity, and stem and bud colour have followed passively as correlated traits (indirect selection). As seen in Table 1, the statistical predictions of this hypothesis (H_{1B}) are identical to the previous hypothesis (H_{1A}). This hypothesis seems considerably less likely, however, because pink and purple bracts do not appear to differ from white and pale green in their attraction of pollinators, as noted above. It is also possible that both floral bracts and vegetative structures are under the same selective pressure for protection from photo-damage, drought stress, chilling or herbivores (selective covariance; Armbruster & Schwaegerle, 1996). Thus, although pollinators do not respond to flower-colour differences, such differences may still reflect response to direct selection. This alternative hypothesis is treated here as less parsimonious than simple pleiotropy (see Methods), but cannot be excluded except through careful experimentation (see Conclusions and future research, below).

The above results do not preclude some floral-colour evolution in *Dalechampia* in response to pollinators. For example response to parallel selection for showiness may

have caused populations in lineages synthesizing anthocyanins to evolve from green to pink styles or staminate sepals, whereas taxa in lineages not synthesizing anthocyanins have evolved from green to white or yellow styles or staminate sepals (Armbruster, 1996). It is also possible that some bract-colour variation within lineages not producing anthocyanins could be the result of direct selection on bract colour. For example, there is a weak tendency for euglossine-pollinated species to have white bracts and megachilid-pollinated species to have green bracts ($P = 0.25$ for number of origins, $P = 0.09$ for number of species; Table 2).

Flower-colour evolution in *Acer*

The analyses presented above generally support the correlated evolution of flower colour and autumn leaf colour in *Acer*, and hence potentially a pleiotropic relationship between these two traits. They also support the hypothesis that some flower-colour diversity has arisen through response to parallel selection acting in at least two different genetic contexts (with vs. without pre-existing anthocyanin biosynthetic capacities).

An alternative explanation for the correlated evolution of flower and leaf colour in maples is that flower colour has radiated in response to selection generated by different pollinators, and autumn leaf colour has responded to indirect selection (Table 1). Although this would be an interesting explanation for the evolution of the colour of autumn foliage (but see Hamilton & Brown, 2001), it seems unlikely because leaf colour changed before flower colour ($P < 0.01$), and flower colour tended to be contingent on leaf colour ($P < 0.08$) rather than vice versa (hypotheses H_{2A} vs. H_{2B} ; Tables 1 and 7). Two additional reasons to doubt this hypothesis are because of the much larger investment of pigments into leaves than flowers, and because the pollination ecology of most green-, yellow- and red-flowered maples appears to be generalized and similar, as noted above. It is also possible that both flowers and leaves are under the same selective pressure for protection from photo-damage, draught, chilling or herbivores. Thus although pollinators are all similar, flower-colour differences could reflect response to direct selection. This alternative hypothesis is treated here as less parsimonious (see above) than pleiotropy, but cannot be completely excluded except through careful experimentation (see Conclusions and future research, below).

These observations and analyses suggest that the reddish pigments (anthocyanins) may have evolved as protective pigments (against photo-inhibition or other photo-damage, drought stress, chilling, or possibly herbivores) in leaves of some maple species (as later evidenced in autumn colours; see Graham, 1998). Adaptation along genetic 'lines of least resistance' (Stebbins, 1974; Schluter, 1996) in response to selection by bees and other pollinating insects for floral spectra

contrasting with the green background (see Spaethe *et al.*, 2001) led to red or purple flowers in many of the species already synthesizing anthocyanins and pale green or yellow flowers in those species not already synthesizing anthocyanins. Thus selection for showy flowers in lineages with different pigment-synthesis systems, in combination with a pleiotropic relationship between floral and leaf pigments, may have created some of the diversity in floral colours seen in the genus today. Direct and indirect selection could be contributing factors, but they are neither necessary nor sufficient, respectively, to explain the broad pattern of flower-colour evolution we see.

Flower-colour diversification through divergent indirect selection

Indirect selection on flower colour seems likely in *Ipomoea* because floral pigments are pleiotropically related to defence compounds that affect levels of herbivory (Simms & Bucher, 1996; Fineblume & Rausher, 1997). This may also be the case in *Raphanus raphanistrum* (Brassicaceae; S. Y. Strauss, pers. comm.). Similarly in *Phlox* (Polemoniaceae), floral colours are closely and pleiotropically related to factors affecting growth rates and are likely to respond to indirect selection (Levin & Brack, 1995).

In the present study, it appears that blossom colour may have diversified in *Dalechampia* through indirect selection. Selection for protection of buds, leaves and stems with various pigments may have caused bract colour to track 'passively' the changes in leaf pigmentation. This is supported not only by the tight association across species, but also by the apparent simultaneous origins of the red bracts and stems three times on the estimated phylogeny.

Flower-colour diversification through parallel selection in diverse genetic contexts

In *Acer*, selection generated by a broad group of unspecialized pollinators for greater contrast of flowers from background foliage (Spaethe *et al.*, 2001) may have operated in parallel in two different genetic contexts and thereby led to diversification in floral colour. Lineages not synthesizing anthocyanins in vegetative parts almost never evolved red or purple flowers. In contrast, lineages with anthocyanin synthesis and red autumn leaves had a much higher probability of giving rise to red/purple-flowered species. This suggests that in lineages not already synthesizing anthocyanins, the genetic line of least resistance towards showy flowers led to carotenoid advertisements, whereas in those lineages already synthesizing anthocyanins, it often led to anthocyanin advertisements. The apparently generalized nature of *Acer* pollination ecology (Matsui, 1991; Waser *et al.*, 1996) may allow parallel selection to operate

across genetically distinct lineages, and hence promote the nonadaptive diversification of floral traits. Such a series of evolutionary events may be much less likely under conditions of more specialized pollination (see Johnson & Steiner, 2000).

Genetic context may have also contributed to the diversification of staminate sepal and style colours away from the basal green colour in *Dalechampia*. The data are too few to be analysed quantitatively, but they suggest that selection by pollinators in one lineage already producing anthocyanins may have led to evolution of pink sepals and/or styles in some (but not all) cases. The same selective pressure may have led to the evolution of white sepals, or white or yellow styles, in lineages not synthesizing anthocyanins.

Conclusions and directions for future research

This study should be treated as provisional; it is presented as an example of an approach to studying the role of processes other than natural selection in evolutionary diversification. This study is not the final analysis of flower-colour evolution in *Dalechampia* and *Acer*, let alone angiosperms in general. Many improvements to this study could be made and additional data are needed. One improvement would be to consider flower colour and showiness in light of the specific spectral sensitivities of the pollinating insects (see, for example, Chittka, 1997; Chittka & Waser, 1997; Spaethe *et al.*, 2001). Another significant improvement would be to study the chemistry and molecular genetics of the pigments in both leaves and flowers. Are the pigments in red flowers chemically the same as in red leaves on the same tree, for example? Do they share common biosynthetic pathways and do they have the same genetic basis, as required by the pleiotropy hypothesis? Finally, with respect to *Acer*, much more information on pollination and phylogenetic relationships of additional species will be necessary for a definitive analysis.

The hypotheses generated by these analyses of macroevolutionary patterns can be tested directly with experiments conducted at the population level. Traits hypothesized to have evolved by indirect selection can be tested for effects on fitness using phenotypic selection analysis (Lande & Arnold, 1983; Wilson & Thomson, 1996) in combination with trait manipulation (e.g. Nilsson, 1988). For example, flower colour can be manipulated or different genotypes used, to see if colour affects visitation rates or constancy (e.g. Waser & Price, 1981, 1983; Levin, 1985; Epperson & Clegg, 1987b). Similar studies of traits hypothesized to be under direct selection can also be conducted using natural or artificial variation in the trait. Improvements in our understanding of the adaptive significance of anthocyanins in vegetative structures are needed and can also be obtained

in this way. Genetic studies employing molecular markers [e.g. quantitative trait loci (QTL) analysis] could be used to test for, and estimate, the strength of the genetic relationships between traits inferred to be pleiotropically related.

The parallel selection hypothesis can be tested by using similar phenotypic-selection studies to assess if both lineages hypothesized to be under the same selective pressure are indeed so, and if the alternate character states (e.g. red vs. yellow flowers) similarly enhance fitness over the ancestral condition (e.g. green flowers). More specific studies of insect responses to colour could address whether alternate colours are indeed equivalently contrasting against the foliage background (Spaethe *et al.*, 2001). Finally, the chemical and molecular-genetic bases for the divergent phenotypes could be investigated to assess whether the genetic basis for flower-colour differences are sufficiently distinct to pose a barrier to, say yellow-flowered lineages evolving red flowers, or vice versa.

This study underscores the caution expressed elsewhere (e.g. Ollerton, 1998; Armbruster *et al.*, 2000) that one should not infer the occurrence of pollinator-induced adaptive radiation from observing variation in flower colour of related species. For example, invoking diverse classes of pollination in *Dalechampia* on the basis of blossom colour (e.g. Webster & Webster, 1972) is apparently erroneous. Instead, the data presented here suggest that flower colour is commonly genetically correlated with other traits, and flower-colour evolution may be influenced by such pleiotropic relationships.

This study demonstrates the potential 'creative' role of pleiotropy and other 'accidental' conditions in generating biological diversity. It illustrates the advantage of taking a broad view of possible sources of intra- and interspecific variation in phenotypic traits, one that includes (1) divergent indirect selection and (2) adaptation to a single selective pressure in diverse genetic contexts, as well as the better studied processes: (3) divergent direct selection and (4) genetic drift. However, much more research is needed to evaluate the relative importance and frequency of these processes in generating diversity in flower colour and other traits. Indeed, many additional studies that explicitly focus on the contribution of nonadaptive processes to biological diversity are required before we can achieve a comprehensive understanding of the sources of biological diversity and, more generally, the evolutionary process.

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