Adaptive evolution of reproductive and vegetative traits driven by breeding systems

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Summary

- The evolution of inflorescence size, a key trait in reproductive success, was studied in the genus *Acer* under a perspective of adaptive evolution. Breeding systems, hypothesized to indicate different levels of mating competition, were considered as the selective scenarios defining different optima of inflorescence size. Larger inflorescences, which increase male fitness by generating larger floral displays, were hypothesized to be selected under scenarios with higher competition with unisexuals. An identical approach was used to test if the same selective regimes could be driving the evolution of leaf size, a vegetative trait that was found to be correlated with inflorescence size.
- A Brownian motion model of inflorescence/leaf-size evolution (which cannot distinguish between changes caused by pure drift processes and changes caused by natural selection in rapidly and randomly changing environments) was compared with several adaptive Ornstein–Uhlenbeck (OU) models, which can quantify the effects of both stochasticity and natural selection.
- The best-fitting model for inflorescence/leaf-size evolution was an OU model with three optima that increased with the level of mating competition.
- Both traits evolved under the same selective regimes and in the same direction, confirming a pattern of correlated evolution. These results show that a selective regime hypothetically related to the evolution of a reproductive trait can also explain the evolution of a vegetative trait.

Key words: *Acer*, adaptive evolution, correlated evolution, inflorescence size, leaf size, mating competition.

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Introduction

An overwhelming diversity in the design of reproductive traits has arisen in flowering plants as a consequence of natural selection improving sexual reproduction. Breeding systems can be considered as selective scenarios modelling these reproductive characters. In angiosperms, for example, some reproductive traits, such as few-flowered inflorescences, evolved under the selective regime that the dioecious condition imposed once this breeding system was already established (Vamosi *et al.*, 2003).

A key trait of angiosperm reproductive success is the inflorescence design, which involves the size, arrangement and phenology of flowers (Wyatt, 1982). The study of the inflorescence has moved from a historical floricentrism towards a more expanded perspective integrating ecological, genetic and evolutionary views (Harder *et al.*, 2004). Two hypotheses on the evolution of inflorescence size have emerged under this broadening perspective: the pollen donation and the plants' dilemma hypotheses (Finer & Morgan, 2003). In the former, large inflorescences are selected because they increase male reproductive success (Broyles & Wyatt, 1990; Burd & Callahan, 2000). While female fitness tends to be limited by resources, male fitness is usually dependent on mating opportunities or pollinator attraction in entomophilous species (Bateman, 1948; Wilson *et al.*, 1994). Thus a larger inflorescence may lead to a larger floral display, which may increase male fitness by attracting more pollinators (Ashman & Hitchens, 2000). The second hypothesis (Klinkhamer & de Jong, 1993) emphasizes that inflorescence size reflects a trade-off (plants' dilemma) between pollinator attraction selecting for large inflorescences and geitonogamy selecting for smaller inflorescences, as a response to reduce the deleterious effects that selfing may cause.

Ackerly & Donoghue (1998) analysed correlated evolution between traits in the genus *Acer* and suggested that the complex arrays of breeding systems within the genus could be the selective scenario shaping the variability of inflorescence sizes. The evolution of breeding systems in *Acer* involved a gradual invasion of unisexual individuals to ancestral monoecious populations (de Jong, 1976). These unisexual mutants could invade and establish successfully only if they had a greater fitness than the pre-existing individuals (Charlesworth, 1999). *Acer* populations were first invaded by male mutant forms (de Jong, 1976). As noted above, male fitness may be increased with larger floral displays. Larger floral displays within this genus are a product of an increase in the number of flowers with inflorescence size, as suggested by Chang & Kim (2003), who found a positive correlation between number of flowers and length of inflorescences. As nearly all the species in the genus are entomophilous (de Jong, 1976), the greater fitness of mutants could probably have been achieved by larger floral displays generated by larger inflorescences, which could have produced an increase in fitness by the attraction of more pollinators. The invasion of the unisexual mutants with larger inflorescences established a situation of intraspecific competition for mating, which should favour the selection of larger inflorescences in all the other individuals.

Duodichogamy (a sequence of male–female–male flowering) has been proposed as the breeding system of the ancestors of the genus *Acer*, based on the breeding system of the unique sister group *Dipteronia* (de Jong, 1976). Heterodichogamy (a breeding system with two reciprocal morphs: male first- and female first-flowering individuals) also appeared in the earliest members of the genus *Acer* (Gleiser & Verdú, 2005). The ancestral populations were then invaded by unisexual forms (males and females) giving rise to polymorphic breeding systems (Fig. 1). This invasion of unisexual forms resulted in the dioecious condition of derived species (de Jong, 1976; Gleiser & Verdú, 2005). It must be noted that geitonogamy is avoided in this genus by a temporal segregation of the sexual phases (dichogamy), so the trade-off in the evolution of inflorescence size mentioned above does not exist in the genus, making pollinator pressure the strongest selective force acting on inflorescence architecture (Harder *et al.*, 2004).

Natural selection on some traits can have further effects on others. As Ackerly & Donoghue (1998) point out, marked patterns of correlated evolution are found among traits at various levels, and interactions can exist among vegetative and reproductive evolution. They found an evolutionary correlation between leaf and inflorescence size in the genus *Acer*, and suggested that the evolution of inflorescence size as a consequence of different selective regimes, imposed by different breeding systems, may drive the evolution of other vegetative traits such as leaf size.

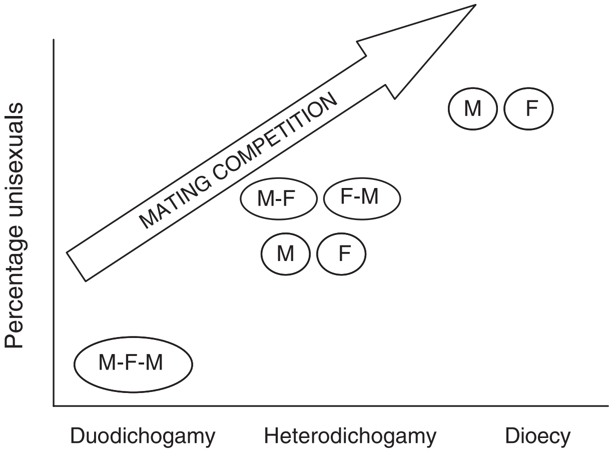

Fig. 1 Breeding systems in *Acer* are characterized by the relative proportion of unisexuals invading monoecious populations (Gleiser & Verdú, 2005). This proportion increases from duodichogamy to dioecy, with heterodichogamy in between. M and F indicate the sexual phase expressing in each individual. As unisexuals could invade the monoecious population successfully only if they had greater fitness than pre-existing individuals (Charlesworth, 1999), then a situation of growing intraspecific competition for mating is predicted in the direction pointed by the arrow.

Studies of correlated evolution of traits among species face the problem of nonindependence of the species because of phylogenetic relatedness (Harvey & Pagel, 1991). Recent advances in comparative biology have allowed the incorporation of phylogenetic information in comparative analyses (Verdú & Traveset, 2005). However, the most popular methods for phylogenetic comparative analysis assume a neutral model of evolution (Brownian motion), which is not able to distinguish between changes caused by pure drift processes from changes caused by natural selection in rapidly and randomly changing environments (Felsenstein, 1988; Hansen & Martins, 1996; Diniz-Filho, 2001). A fundamental improvement in comparative analyses was introduced by Hansen (1997), who proposed modelling evolution through the Ornstein–Uhlenbeck (OU) process. This model has traditionally been interpreted as a model of evolution by genetic drift and stabilizing selection (Lande, 1976), and has been reinterpreted as an adaptation–stochastic selection model (Hansen, 1997). It consists of a simple linear model that allows quantification of both the effects of natural selection and stochasticity (including drift, unconsidered selective factors, random mutation, etc.) (Hansen, 1997; Butler & King, 2004). In addition, this approach provides a powerful tool to estimate multiple selective optima of a trait (see Hansen *et al.*, 2000 for an application of the method to the evolution of *Dalechampia* inflorescence size). Butler & King (2004) have recently developed a mathematical tool to estimate Hansen model parameters and to discriminate among alternative evolutionary hypotheses which can include multiple optima.

The objective of this work is to test if a key reproductive trait (inflorescence size) in the genus *Acer* is modelled by natural selection under a selective regime of competition for reproductive assurance. We tested alternative selective regimes of mating competition according to the different level of invasion of unisexual individuals into the populations (Fig. 1). In addition, we tested the hypothesis that the same selective regimes could be driving the evolution of a vegetative trait (leaf size) that is correlated with inflorescence size.

Materials and Methods

We performed a comparative study of the adaptive evolution of inflorescence size and leaf size under the OU process (Hansen, 1997; Butler & King, 2004). This method requires a data set describing the distribution of a continuous character on a group of species, a clock-like phylogeny with branch lengths, and the construction of one or more hypotheses related to the ancestral selective regimes modelling the study character. These hypotheses are represented by different models, which are constructed by assignment of the hypothetical selective regimes to the internal branches of the phylogeny. The goodness of fit of the different models to the data is finally tested.

We used the pruned phylogenetic tree of Ackerly & Donoghue (1998) containing the 17 *Acer* species in which morphological measures were recorded (Fig. 2). The authors pruned the tree to restrict the ecological component of the study to understorey maples, because the study traits exhibit considerable plasticity in relation to light environments. We estimated the branch lengths of the phylogeny as the number of reconstructed changes occurring in the DNA sequence data matrix using the Trace-All-Changes option in MACCLADE 4.06 (Maddison & Maddison, 2003). All the possible branch length calculations

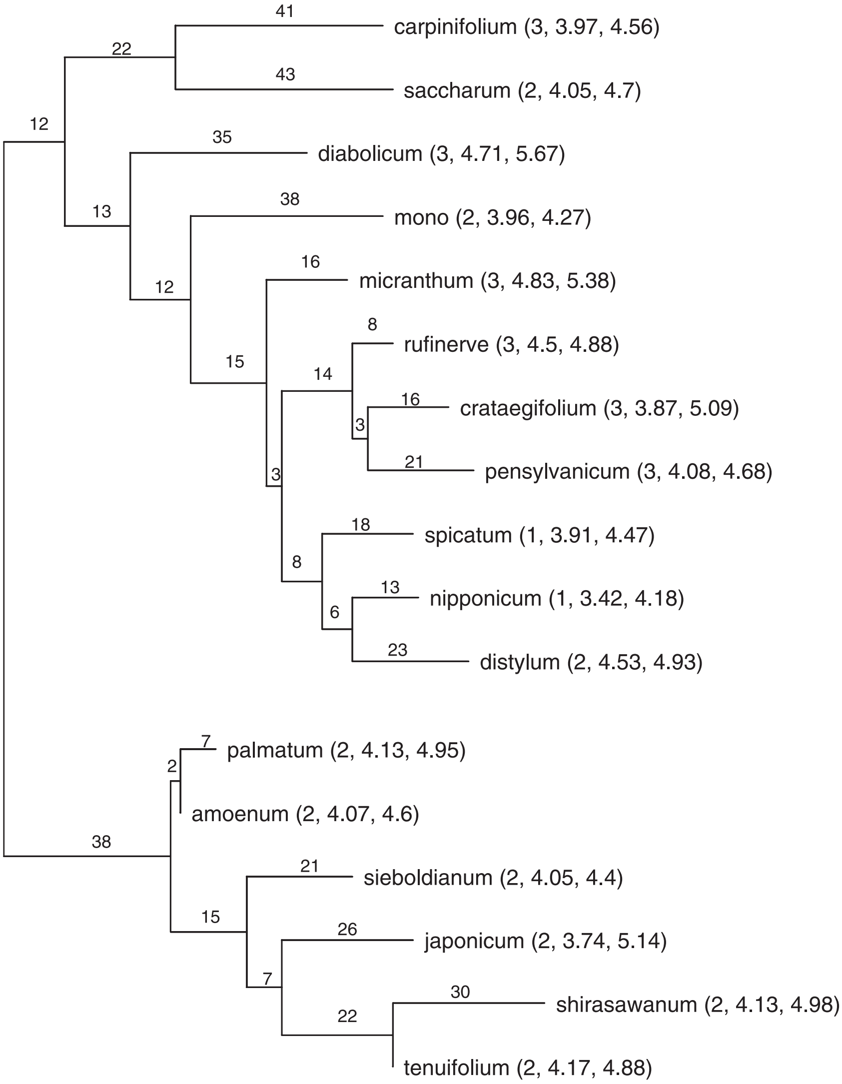

Fig. 2 Original phylogenetic tree and raw data used to test the adaptive evolution of inflorescence and leaf size. Numbers above branches indicate branch lengths estimated as the number of reconstructed changes occurring in the DNA sequence data matrix. Tips of tree show specific name of taxon followed by parentheses containing values of breeding system (1, duodichogamy; 2, heterodichogamy; 3, dioecy); natural logarithm of inflorescence size (mm); and natural logarithm of leaf size (mm).

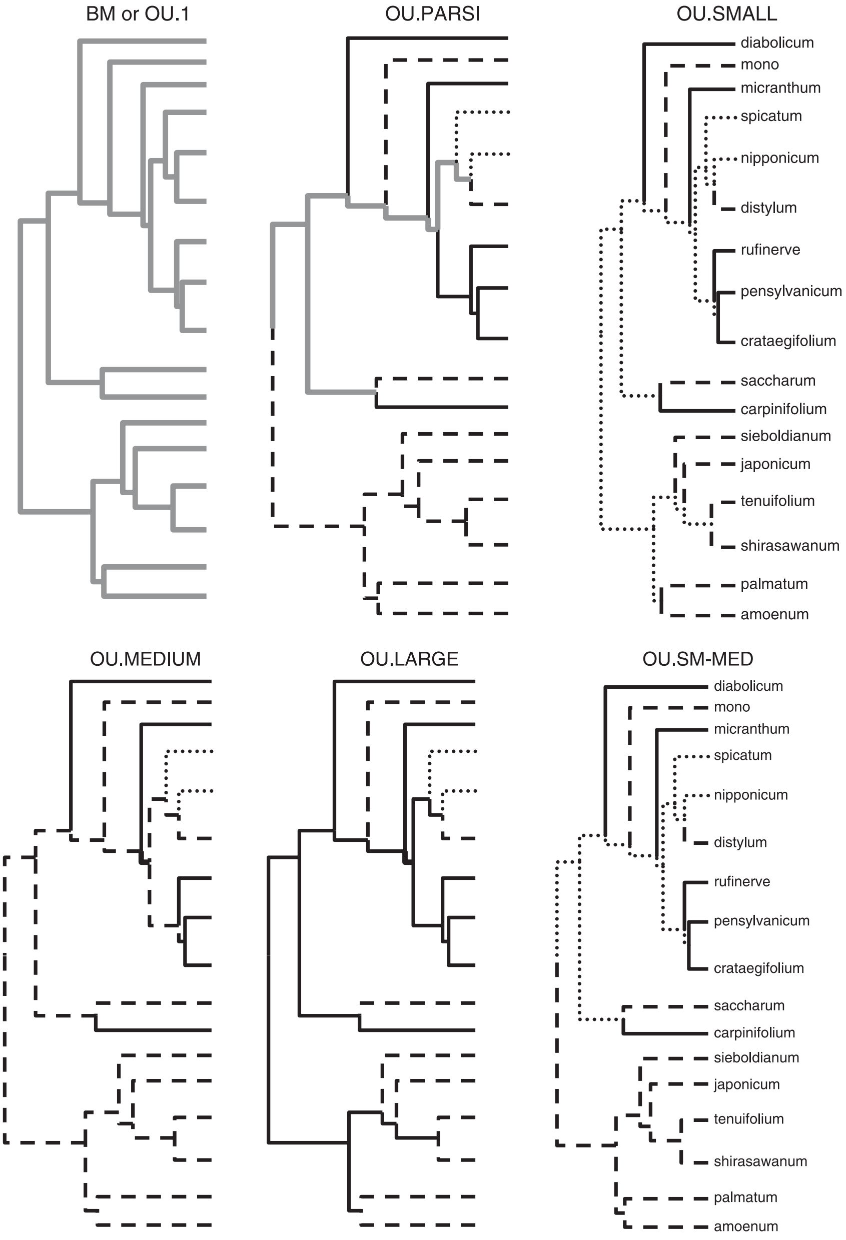

Fig. 3 Ultrametric trees with alternative models describing different selective regimes of mating competition for the evolution of inflorescence size in *Acer*. Breeding systems of *Acer* species (Fig. 2) are assumed to correspond to different selective regimes of mating competition. BM, Brownian motion; OU, Ornstein–Uhlenbeck with different number of optima and ancestral selective regimes (OU.1 assumes a single optimum without inference on ancestral regime; OU.PARSI assumes three optima for each competition regime plus another, unknown, optimum for the ancestral regimes equivocally reconstructed by parsimony; OU.SMALL, OU.MEDIUM and OU.LARGE assume three optima for each competition regime and assume the ancestral regime was under low, intermediate or high competition, respectively). OU.SM-MED assumes three optima for each competition regime and assumes (following the hypotheses of ancestral duodichogamy or heterodichogamy) that the ancestral regime was under low competition for the upper clade and intermediate for the lower clade (see Materials and Methods). Lines in the phylogenetic trees indicate the following mating competition selective regimes: dotted line, low; dashed line, intermediate; solid black line, large; solid grey line, unknown.

implemented in MACCLADE 4.06 (minimum, approximate maximum and maximum number of changes) were done, and the results of the subsequent tests were the same; then we arbitrarily chose the approximate maximum number of changes as the measure of branch lengths in the phylogeny.

Two key traits were also selected from Ackerly & Donoghue's (1998) for the study of adaptive evolution: inflorescence size (a reproductive trait measured as inflorescence + peduncle length); and leaf size (a vegetative trait measured as leaf + petiole length). Both traits were log-transformed.

As we were comparing contemporaneous taxa, we forced our tree to be ultrametric (equal distances from root to all tips) by applying the nonparametric rate-smoothing algorithm described by Sanderson (1997). The branch lengths of this tree are clock-like, and scaled so that the root node has age 1 ($T = 1$). The ultrametric transformation was done in the APE package for R (Paradis & Claude, 2002).

The following models (Fig. 3) reflecting hypothetical selective regimes on inflorescence size were tested:

Model 1, Brownian motion (BM): inflorescence size evolves following a pure drift process and natural selection in rapidly and randomly changing environments.

Model 2, Ornstein–Uhlenbeck with a global optimum (OU.1): inflorescence size evolves under stochasticity and natural selection towards a single global optimum.

Model 3, Ornstein–Uhlenbeck with four different optima reconstructed by parsimony (OU.PARSI): inflorescence size evolves under stochasticity and selection towards four different optima. Three of these four optima correspond to the different selective regimes imposed by the level of mating competition according to the breeding system (low for duodichogamy; intermediate for heterodichogamy; high for dioecy). The fourth optimum was depicted as an unknown ancestral regime because of the equivocal tracing obtained in the parsimony reconstruction. Reconstruction of the breeding systems was done using the MESQUITE 1.04 program (Maddison & Maddison, 2004), and the result was the same when considering the character either ordered or unordered.

Models 4 (OU.SMALL), 5 (OU.MEDIUM) and 6 (OU.LARGE): similar to OU.PARSI, but assuming the adaptive regime of the internal branches corresponds to a low (as in duodichogamy); intermediate (as in heterodichogamy); or high (as in dioecy) level of mating competition, respectively.

Model 7, OU.SM-MED: assigns the two proposed ancestral breeding systems (duodichogamy and heterodichogamy, de Jong, 1976; Gleiser & Verdú, 2005) to the internal branches of the phylogeny, allowing one clade fully represented by heterodichogamous species (lower clade in Fig. 2) to evolve under intermediate competition, and the other (upper clade in Fig. 2) to evolve under the regime of low competition represented by the duodichogamous state.

To test if the mating competition selective regime is also driving the evolution of a vegetative, related trait, we repeated the same analyses for leaf size.

The BM model is a special case of (is nested within) OU models because, if we assume that the rate of adaptation (α) is zero, OU collapses to BM. Thus we compared OU against BM models by means of the likelihood ratio test assuming a χ^2 distribution. Similarly, we compared the single-optimum OU model (OU.1) with the multiple-optima OU models. When the models are nonnested (as in the comparisons between OU candidate models with identical degrees of freedom), the χ^2 approximation is no longer valid, and we then used a different approach to model selection, the minimization of the Akaike information criterion (AIC) (Posada & Buckley, 2004). We accounted for the model selection uncertainty with the AIC by estimating the Akaike weights to calculate a model-averaged estimate of parameters σ (magnitude of the stochasticity component); α (rate of adaptation); θ_0 (estimated ancestral value for the most basal node of the tree); and θ_i (optima values estimated for each selective regime), as described by Posada & Buckley (2004).

We computed the confidence intervals of the parameters in the individual models using parametric bootstrap with 10 000 replicates. All analyses were done in the OUCH package for R (Butler & King, 2004).

Results and Discussion

The magnitude of the stochasticity component (σ) experienced by inflorescence size within the genus *Acer* was similar for BM and OU models, as all the confidence intervals overlapped (Table 1). However, the greatest difference appeared once we estimated the rate of adaptation (α): all the OU models showed that selection is a strong force significantly different from zero. For this reason, the BM model of inflorescence size evolution was outperformed by all OU models (likelihood ratio (LR) tests against BM in Table 1), indicating that inflorescence size evolved because of selection towards one or several optima.

One indication of the large magnitude of the adaptation rate is the low dependence of traits of extant species on the value of the root (Butler & King, 2004). This dependence is the conditional expectation of the phenotypic value at the end of the evolutionary process, and can be calculated as $e^{(-\alpha T)}$ (where α is the rate of adaptation and T is the age of the root node), which represents a linear function of the root ancestral state and the optima along each tree segment in the lineage, weighted by the strength of selection (see Butler & King, 2004 for mathematical details). In our case, this dependence is close to zero [$e^{(-\alpha T)} = e^{(-11.49)(1)} = 0.001\%$]. If the rate of adaptation (α) is large, then the trait values at the tree tips provide insufficient information to allow us to estimate the ancestral value at the root of the tree (θ_0 in Tables 1 and 2). So the inability of the equation to estimate the ancestral value is a clear manifestation of the strong selection acting on inflorescence size (A. A. King, personal communication).

All the multiple-optima OU models performed much better than the single-optimum model (LR tests against OU.1 in Table 1), indicating that inflorescence size evolution has been driven to different optima by the selective regimes imposed by the breeding systems.

The parsimony reconstruction model (OU.PARSI) had a low performance (large AIC values and therefore small Akaike weights in Tables 1 and 2), probably because of equivocal tracings towards the base of the tree. Similarly, Gleiser & Verdú (2005) found an equivocal reconstruction of the ancestral breeding system in *Acer*, where duodichogamy (low competition level) and heterodichogamy (intermediate competition level) have been proposed as the putative ancestral states. Our data do not provide evidence to resolve this equivocal tracing of the breeding system, because the algorithm failed to estimate the ancestral value for the most basal node of the tree in all the OU models. According to this uncertainty towards the root of the tree, the models with greater weights were those in which the ancestral selective regimes depict low and medium competition levels (OU.SM-MED; OU.SMALL; OU.MEDIUM) (see Akaike weight values in Table 1). The model representing a dioecious-like, high-competition, ancestral selective regime (OU.LARGE) was the worst (Table 1), supporting the reconstruction of dioecy as a derived, not ancestral, breeding system in the genus (Gleiser & Verdú, 2005).

All the OU models showed an increase in inflorescence size optima as the level of mating competition increased (Table 1), supporting our hypothesis which argues that higher floral displays are selected under situations of higher competition with

Table 1 Performance and parameters estimated for seven alternative models describing inflorescence size evolution in *Acer*: model performance showing likelihood values, Akaike information criterion (AIC), Akaike weight, degrees of freedom and *P* values of likelihood ratio tests comparing each model against the BM and OU.1 models, and parameters of the models

	BM	OU.1	OU.PARSI	OU.SMALL	OU.MEDIUM	OU.LARGE	OU.SM-MED	Model-averaged estimate
Performance								
$-2 \log L$	23.61	11.61	3.84	3.55	3.80	4.35	3.38	–
AIC	27.61	19.61	17.84	15.55	15.80	16.35	15.38	–
Akaike weight (%)	–	–	8.04	25.25	22.29	16.93	27.49	–
df	2	4	7	6	6	6	6	–
LR *P* value against BM	–	0.002	0.001	0.0005	0.0005	0.0007	0.0004	–
LR *P* value against OU.1	–	–	0.05	0.01	0.02	0.02	0.01	–
Parameters								
α	–	62.7	17.1	12.5	8.9	10.2	11.8	
		(0.90, 99.8)	(1.20, 48.8)	(2.69, 47.8)	(0.73, 64.4)	(0.71, 54.8)	(2.89, 48.3)	11.5
σ	0.65	3.81	1.59	1.34	1.15	1.24	1.30	
	(0.41, 0.85)	(0.46, 5.02)	(0.36, 2.35)	(0.48, 2.38)	(0.33, 2.74)	(0.34, 2.52)	(0.49, 2.37)	1.29
θ_0	4.14	a	a	a	a	a	a	–
	(3.48, 4.72)							
θ_{global}	–	4.12	–	–	–	–	–	–
		(3.93, 4.90)						
θ_{small}	–	–	3.50	3.66	3.57	3.55	3.66	3.61
			(0.74, 4.01)	(3.24, 4.04)	(0.45, 4.04)	(0.41, 4.04)	(3.25, 4.00)	
θ_{medium}	–	–	4.07	4.10	4.08	4.07	4.09	4.09
			(3.69, 4.33)	(3.90, 4.33)	(3.79, 4.45)	(3.77, 4.37)	(3.90, 4.29)	
θ_{large}	–	–	4.32	4.41	4.41	4.35	4.42	4.40
			(3.99, 4.69)	(4.16, 4.79)	(4.07, 5.03)	(4.10, 4.74)	(4.17, 4.85)	
$\theta_{unknown\ ancestral\ regime}$	–	–	a	–	–	–	–	–

Parameters : α, rate of adaptation; σ, magnitude of stochasticity component; θ_0, estimated ancestral value for most basal node of tree; θ_k (where k indicates global, small, medium, large or unknown ancestral regime), optima estimated for different selective regimes.
95% Confidence intervals are shown below each parameter.
a, Algorithm failed to estimate a parameter (Butler & King, 2004). LR, likelihood ratio.

unisexual forms for reproduction. Despite the slight overlap in the 95% CI, the trend, which is consistent in all the models, suggests biologically important information. Unisexual forms are usually super-specialized in one of the sexual functions, outcompeting the same function of the monoecious individuals (Verdú, 2004; Verdú *et al.*, 2004a). According to this, the sexual specialization of males in some *Acer* species studied allows them to hoard most of the male function in the population, relegating the fitness gain of monoecious individuals to the female function (Sato, 2002; Verdú *et al.*, 2004b; G.G., M.V. and J.R. Pannell, unpublished data).

The model-averaged estimates of the different optima indicate that the optimum size of inflorescences at low competition level is stabilized around 37 mm (back-transformation of the natural logarithm of 3.61 mm in Table 1). Once the competition for mating has increased because of the invasion of unisexuals, the inflorescence evolves towards a second optimum around 60 mm and, finally, the total invasion of unisexuals (dioecy) pushes the size of inflorescences up to the higher optimum at 81 mm. The time it took a species to move half the distance to its optimum (adaptive half-life *sensu* Hansen, 1997) can be estimated as $\ln(2)/\alpha$, yielding a value of approx. 60 000 yr. It should be noted that this estimation assumes that time relies on clock-like evolution of the molecular characters used to reconstruct the phylogeny, which usually does not occur in undersampled phylogenies (Linder *et al.*, 2005). To make the rates of adaptive evolution comparable across future studies, absolute time scales obtained by means of well calibrated phylogenies will be necessary.

For this adaptive scenario to be true under the pollen-donation hypothesis, large inflorescences must increase male reproductive success (Burd & Callahan, 2000). It is conceivable that this occurs in *Acer*, where (1) larger inflorescences contain more flowers than smaller ones (Chang & Kim, 2003); and (2) the number of pollinator visits increases with number of male flowers (G.G., M.V. and J.R. Pannell, unpublished data). It should be noted that other selective, unknown factors may be also acting in the evolution of inflorescence size. As noted above, the OU model is a linear function with two components: one involving stabilizing selection, the other reflecting stochastic perturbations such as those caused by the effect of unconsidered selective factors or those caused by genetic correlations with other traits, environmental fluctuations, and even genetic drift and random mutations (Hansen, 1997).

Table 2 Performance and parameters estimated for seven alternative models describing leaf size evolution in *Acer*

	BM	OU.1	OU.PARSI	OU.SMALL	OU.MEDIUM	OU.LARGE	OU.SM-MED	Model-averaged estimate
Performance								
Likelihood	20.60	15.19	8.19	7.81	7.50	7.55	7.35	–
AIC	24.60	23.19	22.19	19.80	19.50	19.50	19.35	–
Akaike weight (%)	–	–	6.20	20.50	23.81	23.81	25.67	–
df	2	4	7	6	6	6	6	–
LR *P* value against BM	–	0.06	0.03	0.01	0.01	0.01	0.01	–
LR *P* value against OU.1	–	–	0.07	0.02	0.02	0.02	0.02	–
Parameters								
α	–	116.7	4.12	13.8	4.94	4.33	9.94	
		(1.09, 136.29)	(0.83, 43.79)	(2.31, 53.60)	(0.62, 54.38)	(0.51, 36.53)	(2.57, 41.85)	7.84
σ	0.60	5.78	0.89	1.60	0.95	0.90	1.34	
	(0.38, 0.78)	(0.52, 5.85)	(0.36, 2.30)	(0.51, 2.68)	(0.36, 2.63)	(0.35, 2.16)	(0.51, 2.29)	1.17
θ_0	4.83	a	a	a	a	a	a	–
	(4.23, 5.36)							
θ_{global}	–	4.81	–	–	–	–	–	–
		(4.63, 5.27)						
θ_{small}	–	–	4.08	4.32	4.07	3.83	4.32	4.12
			(–0.18, 5.13)	(3.87, 4.81)	(0.72, 4.92)	(–1.08, 4.72)	(3.85, 4.75)	
θ_{medium}	–	–	4.83	4.77	4.78	4.71	4.77	4.76
			(3.19, 5.14)	(4.54, 5.06)	(3.70, 5.17)	(3.02, 5.03)	(4.56, 5.03)	
θ_{large}	–	–	5.25	5.16	5.24	5.16	5.16	5.18
			(4.35, 5.92)	(4.82, 5.61)	(4.78, 5.98)	(4.67, 5.66)	(4.85, 5.72)	
$\theta_{unknown\ ancestral\ regime}$	–	–	4.73	–	–	–	–	–
			(–48.63, 49.91)					

Parameters : α, rate of adaptation; σ, magnitude of stochasticity component; θ_0, estimated ancestral value for most basal node of tree; θ_k (where *k* indicates global, small, medium, large or unknown ancestral regime), optima estimated for different selective regimes.

95% Confidence intervals are shown below each parameter.

a, Algorithm failed to estimate a parameter (Butler & King, 2004). LR, likelihood ratio.

Nevertheless, as Hansen (1997) points out, the dominant mode of evolution of phenotypic quantitative characters is maintenance by stabilizing selection, and the stochastic component arising in the model is probably caused by the influence of other selective factors not included in the analysis.

A decreasing evolutionary trend in inflorescence size has been suggested to occur in *Acer* (de Jong, 1976). An evolutionary trend is produced when the average value of a trait changes directionally within a clade (Verdú, 2005). For a decreasing trend to occur, it is necessary that the *Acer* ancestral inflorescence is large. However, our data do not support this hypothesis because the model representing large-sized inflorescence optima in the ancestral branches of the phylogeny (OU.LARGE) has little support. de Jong (1976) suggested different morphological trends in the genus *Acer*, assuming a directional trend from duodichogamy to dioecy that has been questioned recently (Gleiser & Verdú, 2005).

Leaf size was also found to have evolved under natural selection towards one or several optima. Again, all the OU models fitted the data significantly better than the BM model (LR tests in Table 2). It is remarkable that the OU model with the best performance was again the OU.SM-MED model (Table 2). In addition, the association between selective regimes and leaf size was the same as that found for inflorescence size, because larger leaves have been selected in scenarios of increasing levels of competition (optima values in Table 2). Thus a selective regime hypothetically related to the evolution of a reproductive trait can also explain the evolution of a vegetative trait. The most parsimonious explanation is that reproductive and vegetative traits are evolutionarily correlated, and therefore the increase of inflorescence size as a result of high levels of competition for mating is producing a concomitant increase in leaf size. This concomitant change between inflorescence and leaf size is biologically meaningful as long as larger inflorescences require larger leaves to produce further resources (Ackerly & Donoghue, 1998). A biomechanical alternative hypothesis would also explain the link between inflorescence and leaf size if there is a trade-off between branch size and spacing (selection for large inflorescences implies that branch tips have to be further apart, so large leaves are favoured to fill the space geometrically). Both resource supply and biomechanics hypotheses lead to the conclusion of correlational selection.

Correlational selection is the most plausible of the scenarios (indirect selection; parallel selection; correlational selection: Armbruster, 2002; Pausas & Verdú, 2005) proposed to explain correlated evolution. Under correlational selection we would expect natural selection acting not only on the means and variances of inflorescence and leaf size, but also in the covariance of both traits, and consequently a selection-mediated phenotypic integration of vegetative and reproductive traits (*sensu* Herrera, 2001). Thus the positive correlation between the evolution of inflorescence size and leaf size found by Ackerly & Donoghue (1998) by means of a Brownian model can be confirmed under a broader evolutionary model. Other studies on *Acer* have also shown a significant evolutionary correlation between reproductive (floral colour) and vegetative (leaf colour) characters (Armbruster, 2002). However, the evolution of phenotypic covariance structure in plants may be more complicated, with some floral traits tending to covary with vegetative traits more than other floral traits (Armbruster *et al.*, 1999).

In conclusion, inflorescence and leaf-size evolution in *Acer* are best described by adaptive models with several optima. In addition, the evolution of leaf size and inflorescence size is best described by the same adaptive model, thus both traits evolved under the same selective regimes and in the same direction, confirming a pattern of correlated evolution.

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