

Editorial

Darwinism renewed: contemporary studies of plant adaptation

One hundred fifty years after *On the origin of species* was first published (Darwin, 1859), biologists continue to share Darwin's fascination with the adaptive traits of organisms, and to theoretically and empirically expand his initial formulation of natural selection as the process that shapes those traits. In this special issue of the journal, *New Phytologist* recognizes this continuing legacy by bringing together current ideas and findings about plant adaptation from an accomplished, multi-national group of researchers studying a wide array of plant systems. Many of these studies make use of the formidable set of research tools now available to examine the molecular and genomic bases of adaptive traits and their selective dynamics, and these tools have certainly provided critical insights. But the most exciting aspect of contemporary work on adaptation is not these new techniques *per se*, but rather the way researchers are combining diverse tools in robust, richly informative interdisciplinary approaches. Genomic data from model organisms are being used in new ways to inform studies of naturally evolved systems, and a rigorous phylogenetic context has become standard across sub-disciplines to refine population, species, and higher-level comparisons.

The studies presented here address questions at all levels from the molecular to the macroevolutionary, drawing on information ranging from transgenic functional assays, transcriptomics, and quantitative genetics trait matrices to GIS-based niche modeling, field explant studies of recombinant inbred lines, and distribution data from herbarium sheets. This new work makes clear how these sophisticated interdisciplinary approaches are illuminating some of the most fundamental and long-standing questions about plant adaptation and the process of selective change. It also reflects an increasing awareness that the study of adaptation has important bearing on biodiversity conservation, invasion biology, and potential constraints on adaptive evolution in the face of rapidly changing environments. Here, a brief overview of the feature points to some of the key findings with respect to several areas of shared focus: co-evolutionary interactions; floral and mating system evolution; geographic patterns of adaptive evolution; genetic architecture of adaptation; and evolution of functional traits.

Co-evolutionary interactions

The interactions of plants with their pathogens, parasites and pollinators can generate reciprocal selection pressures and consequently a distinctive co-evolutionary process. L. G. Barrett *et al.* (this issue, pp. 513–529) analyze this process for the complex spectrum of associations between plants and their microbial pathogens. In contrast to the specific, highly virulent pairwise interactions that have been emphasized to date in co-evolutionary models, they argue that host-pathogen associations form a continuum from specialists to generalists, with widely varying fitness effects (and therefore selective outcomes) depending on environmental and genetic factors that jointly influence the expression of both microbial virulence and host resistance genes. These factors can interact at several levels to affect selection pressures, for instance in the case of co-infection by multiple pathogens that interact within the host to modify its defence gene expression. Godsoe *et al.* (pp. 589–599) focus on a very different aspect of plant co-evolutionary dynamics, a well-studied pollination mutualism. Using GIS-based niche modeling and well-resolved phylogenetic data, they determine that the specialized biotic interaction between *Yucca brevifolia* and its moth pollinators, rather than climatic variables, have led to evolutionary divergence in this system.

Floral and mating system evolution

Floral features such as pollination syndromes and organ placement provide clear examples of adaptation. Harder & Johnson (pp. 530–545) comprehensively review evidence as to whether floral and inflorescence traits fit a Darwinian model of gradual, consistent selective change. Although data from manipulation experiments generally confirm that these 'beautiful contrivances' are indeed adaptations that evolved for pollen transfer, the results of phenotypic selection analyses indicate that directional selection on these traits may be relatively weak and inconsistent in natural populations. Instead, adaptive floral features may be shaped largely during episodes of strong selection rather than by continual gradual selection as Darwin envisioned. This meta-analysis reveals limits to phenotypic selection analysis as a definitive test for adaptive function. Armbruster *et al.* (pp. 600–617) pose a complementary question about the evolution of floral adaptations: does floral diversification at the macroevolutionary level reflect adaptation to pollinators? They determine an optimal-fitness 'adaptive surface' relating pollination performance to anther and stigma position, and test against this prediction the realized distribution of floral traits in three phylogenetically diverse genera. Their analysis

reveals clade-specific departures from the optimum reflecting both lack of floral integration and conflicting selection pressures for other outcrossing features. Fenster *et al.* (pp. 502–506) draw our attention to vertical versus horizontal floral orientation as a significant influence on pollinator effectiveness that has been largely ignored for the past century. Their initial dataset shows that hummingbird approach behavior and floral contact vary in response to differently oriented artificial flowers, a result that argues for future pollination studies to include this aspect of floral adaptation.

Gender expression and the evolution of plant mating systems have long been a focus of theoretical and empirical interest. S. C. H. Barrett *et al.* (pp. 546–556) present a well-developed case study on the evolution of selfing based on phylogeographic analysis of multilocus nuclear DNA sequences (SNPs) from populations sampled across the Neotropical range of the sexually polymorphic species *Eichornia paniculata*. Their data reveal multiple independent transitions from outcrossing to selfing in populations of this colonizing species. Preliminary results also suggest that both genetic factors and environmental stress contribute to developmental instability in the early stages of selfing, which may facilitate this evolutionary transition. Randle *et al.* (pp. 618–629) investigate the ecological implications of self-fertilization: does selfing promote colonization and hence range expansion, or does the lower genetic diversity of selfing species instead restrict range size? They test the relation of selfing ability to size of realized geographic range in *Collinsia*, combining a phylogenetically controlled species-pair comparison with precise measures of floral form and self-fertilization activity. This elegant study establishes empirically that species with the highest proficiency for autonomous selfing also have significantly larger ranges, linking an individual reproductive trait with ecological distribution including, as predicted by Herbert Baker, the spread of weedy and invasive taxa. Two additional papers analyze the evolution of mating systems. In one, Mazer *et al.* (pp. 630–648) demonstrate that pollen:ovule ratios are temporally more constant in selfers than in outcrossers, consistent with the expectation that the optimal P:O ratio varies more temporally in outcrossers. Van Etten & Chang (pp. 649–660) test whether the Sex-Differential Plasticity hypothesis – which posits that plasticity in hermaphrodites allows them to reduce seed production in harsh environments, allowing the invasion of females – can explain variation among populations in whether females are present in addition to hermaphrodites in *Geranium maculatum*.

Geographic patterns of adaptive evolution

Patterns of geographic spread and diversification reflect the interplay of gene flow with selective and neutral evolutionary forces across ecological landscapes. Dispersal traits are of central importance to this process, but other aspects of plant phenotypes may play a key role as well. Levin (pp. 661–666) proposes a previously unrecognized connection between individual

plasticity and adaptive divergence of populations. (The paper is graciously dedicated to the late Fakhri A. Bazzaz, whose work deeply enhanced our understanding of plasticity and its ecological implications.) Levin reviews the evolutionary ecology literature to establish that individuals encountering novel conditions often displace the timing of flowering. He argues that, when a population colonizes a new habitat, such an environmentally-induced phenological shift will lead to temporally assortative mating that effectively isolates the colonizing population from its source. As a result, phenological plasticity will facilitate local adaptation to the new habitat that would otherwise be impeded by gene flow from the source population. Along with intriguing implications for ecological range expansion, this paper adds a new dimension to contemporary ideas about the possible role of individual plasticity in evolutionary diversification.

Questions of dispersal and range also hold immediate implications for extinction risks as natural habitats are increasingly disrupted. For instance, effective dispersal can contribute to species persistence in fragmented landscapes by allowing re-colonization of habitat patches and outcrossing. However, if habitat fragmentation in itself imposes selection for reduced dispersal ability (as occurs in island populations), this evolutionary feedback will worsen its negative impact on species' distributions and persistence. Riba *et al.* (pp. 667–677) find a negative correlation between the degree of landscape fragmentation and achene dispersal ability at both local and regional spatial scales in the wind-dispersed European herb *Myrcalis muralis*. Together with a common garden experiment confirming a partial genetic basis to this trait, these results indicate that fragmentation may have negative evolutionary as well as ecological consequences.

Geographic patterns of variation can also provide insights into the spread of invasive species, including the possible role of selective adaptation to the introduced range. Keller *et al.* (pp. 678–690) document differentiation patterns in two weedy European *Silene* species that are rapidly spreading in North America. By characterizing patterns of variation for neutral genetic (AFLP) markers, the authors statistically account for random drift and colonization history to test for the presence of adaptive clinal patterns. In addition to selectively neutral genetic structure, the range expansion of introduced *Silene* spp. also reflects adaptive evolution in response to local environmental gradients. Murren *et al.* (pp. 691–701) examine selective response in non-native populations of *Mimulus guttatus*, finding evidence of selection for larger plant size compared with native populations. Analysis of transects across hybrid zones allows examination of the forces of divergent selection that generates geographic variation and maintains species differences despite gene flow. Using such an approach, Brennan *et al.* (pp. 702–717) report that both intrinsic selection against hybrids and environmentally imposed divergent selection on several traits actively maintain the integrity of two hybridizing species of *Senecio* on Mt. Etna.

Genetic architecture of adaptation

New molecular and analytical tools, and new ways of combining them, have made possible a far more empirically rich understanding of the genetic basis of adaptive evolution. At the same time, a contemporary view of genomes as highly interconnected regulatory systems raises critical questions about the genetic architecture of adaptive traits: what kinds of genetic changes underlie novel adaptive phenotypes, and do these same changes arise repeatedly under similar selective pressures? What potential constraints on adaptive evolution are posed by gene interactions and genetic correlations among traits? One key insight to emerge from the recent explosion of genome and transcriptome data is the pivotal role of gene duplication. Flagel & Wendel (pp. 557–564) argue that this is a primary source of evolutionary innovation in plants, whose highly duplicated genomes reflect past and/or recent polyploid events. They describe the diverse array of gene duplication mechanisms known in plants, and explain the distinct ways they can contribute to novel phenotypes. For instance, transposon-mediated duplications can insert a gene into a new regulatory context that alters its expression, while allopolyploidy allows divergent regulatory systems to instantly become ‘co-resident’ genomes, resulting in higher-order gene interaction networks that can produce major phenotypic shifts. More generally, the genetic redundancy created by these various mechanisms can lead to either the evolution of new adaptive functions or to ‘sub-functionalization’ (division of labor among gene copies), which comprises an evolutionary solution to antagonistic pleiotropy.

The evolution of adaptive traits is empirically explored in several strong case studies. Di Stilio *et al.* (pp. 718–728) explore the evolutionary-developmental basis of floral phenotype in three species of *Thalictrum* with contrasting pollination syndromes, focusing on a transcription factor in the MYB family that is a candidate gene for floral epidermal features related to petal production. Their study illuminates the active evolution of gene lineages, their developmental impact at the cellular level, and the relationship of gene expression to differently adapted pollination syndromes.

Studies of floral color, defense chemicals, and stress tolerance show that diverse genetic mechanisms can underlie repeated evolution of the same adaptive transition. Cooley & Willis (pp. 729–739) show that red color has evolved repeatedly in a monophyletic group of *Mimulus* species via different, unique combinations of Mendelian and polygenic factors that influence petal anthocyanins. In another study of adaptive evolutionary convergence, Neiman *et al.* (pp. 740–750) examine selection on protease inhibitor loci involved in highly specific induced–defense interactions with herbivores. They document surprisingly variable patterns of nucleotide diversity and inferred selective histories within and between *Populus* species for proteins with putatively similar ecological function. Streisfeld & Rausher (pp. 751–763) examine three independent transitions from blue to red flower color in *Ipomoea*. Although

this change could in theory result from a number of possible types of mutation along the well-characterized anthocyanin pathway, their results implicate the same gene in all three cases, and furthermore suggest that regulatory mutations play the primary role in these repeated evolutionary transitions. Dassanayake *et al.* (pp. 764–775) explore the genetic basis of physiological adaptation, presenting the first genomic data for two phylogenetically disparate mangrove species, a system of exceptional ecological interest. They characterize transcriptomes obtained by pyrosequencing, functionally annotating them using *Arabidopsis* and *Populus* genome data. The results show remarkably similar transcriptome profiles in the mangroves, indicating adaptive convergence at the gene expression level. Lowry *et al.* (pp. 776–788) examine the evolution of salt tolerance at the population level. In a comparison of coastal and inland *Mimulus* ecotypes, they identify quantitative trait loci (QTL) involved in salt-spray tolerance and fitness in a coastal site. Interestingly, they found no negative consequences of the alternative QTLs across habitats, suggesting that local adaptation may involve distinct sets of loci that are functionally neutral in other environments.

Kover *et al.* (pp. 816–825) explicitly focus on the genetic architecture of local adaptation: do genetic changes in response to selection in one environment entail negative fitness effects in other conditions? An experimental evolution approach suggests a complex answer. The authors artificially selected for early flowering in *Arabidopsis* in two simulated seasonal regimes, producing distinctly adapted lines. They find that in this system, the genetic basis of life-history adaptation includes *both* alleles with positive effects in both environments, and environment-specific allelic effects that have some negative impact in the alternative treatment. This underlying complexity may act to maintain genetic diversity for fitness-related traits. Muir & Moyle (pp. 789–802) examine a second aspect of genetic architecture that can affect adaptive evolution: the relative phenotypic contributions of additive and epistatic interactions between loci (in this case, target chromosomal regions tested in controlled pairwise combinations in multiple nearly-isogenic lines of *Solanum*). In this system, not only are epistatic effects a major component of total genetic impact on functional traits, most of these non-additive effects are antagonistic. For instance, chromosomal regions that individually decrease water-use efficiency (WUE) restore drought resistance when they occur in combination. These results point to the important conclusion that evaluating the main effect of individual loci may underestimate the loci that influence a complex adaptive trait, and consequently the genetic constraints on adaptive evolution.

Scoville *et al.* (pp. 803–815) focus on the pleiotropic impact of allelic variation as a potential evolutionary constraint. Their study integrates QTL mapping and G-matrix estimation to quantify evolutionary potential of a suite of floral and life-history traits in a natural population of *Mimulus guttatus*. The results of this innovative work exemplify how allelic changes at even

one locus can realign the pattern of genetic covariances for fitness-related traits, altering both constraints to adaptation and levels of standing genetic variation. Galloway *et al.* (pp. 826–838) examine another critical aspect of potential constraints on adaptive evolution: maternal effects. Because maternal and offspring traits are expressed at different times, idiosyncratic trajectories of selective change can occur for traits influenced by these inter-generational effects. Building on an impressive body of work on environmental maternal effects in the forest herb *Campanulastrum americanum*, Galloway and colleagues identify strong effects of maternal genotype on the potential for selective response in offspring traits.

Evolution of functional traits

Papers in this area include both creative interdisciplinary work on the evolutionary history of functional innovations, and studies of the process of selection on functional traits. To begin the section, a provocative review by Sadras & Denison (pp. 565–574) re-evaluates the traditional view among physiologists and crop scientists that growing plant organs compete with one another as alternative 'sinks' for finite resources. They instead propose to consider resource allocation mechanisms in plants within an explicitly evolutionary framework. Such a framework has two components: first, the recognition that any conflict for resources within a genetic individual is selectively constrained by its effect on the fitness of the entire organism; and second, a view of genetically distinct plant structures (such as outcrossed progeny developing on a maternal plant) in the context of selective models for parent-offspring fitness trade-offs and sibling rivalry.

Brodribb and colleagues (pp. 839–847) consider a critical macroevolutionary aspect of ecophysiological adaptation. Their comparative study assesses stomatal control sensitivity to a broad range of CO₂ levels in taxa representing major land plant lineages. The results are striking: in contrast to the sampled ferns, lycopods and gymnosperms, angiosperms have evolved the unique ability to close in response to high CO₂ levels, a response that maximizes water-use efficiency (but reduces carbon fixation) under such conditions. In their 'phylogenetic ecology' study, Agrawal *et al.* (pp. 848–867) examine the evolutionary gain and loss of leaf surface traits important to both ecophysiology and herbivore resistance in the monophyletic, ecologically diverse genus *Asclepias*. Gibson & Waller (pp. 575–587) follow a passionate interest of Darwin's to evaluate the selective pressures that likely promoted the transition from sticky traps to highly modified snap-traps in carnivorous plants, a remarkable functional innovation that evolved just once.

At the population level, Donovan *et al.* (pp. 868–879) evaluate evidence for resource-based natural selection on ecophysiological traits in two distinct dune habitats in desert *Helianthus* taxa. Although they find evidence for direct selection on several functional leaf traits, both the strength and the direction of

selection are highly variable, creating changing temporal and spatial patterns rather than consistent, habitat-specific selective pressures. McGoey & Stinchcombe (pp. 880–891) show empirically that selection on adaptive shade avoidance plasticity in *Impatiens capensis* depends on whether competitors are conspecific or heterospecific, refining our understanding of this important aspect of selection in natural communities. Two papers examine the adaptive relevance of plant morphological structures. Mao & Huang (pp. 892–899) demonstrate that in plants in which pollen is protected from rain by floral structures, pollen is less resistant to water damage than pollen from plants in which pollen is not protected. Wise (pp. 900–907) describes a growth-form polymorphism in *Solidago altissima* in which some individuals, instead of producing erect flowering stems, produce stems that recurve toward the ground. He shows that this alternate growth form reduces attack by apex-galling herbivores, suggesting that it may be maintained in populations by selection imposed by herbivores.

Finally, two papers focus on phenotypic plasticity in physiological traits. Maherali *et al.* (pp. 908–918) consider the question of whether changes in physiological traits over the life cycle are adaptive. They demonstrate that in *Avena barbata*, ontogenic change in photosynthesis is adaptive in wet-soil environments, though apparently neutral in dry soils. Lev-Yadun & Holopainen (pp. 506–512) address a long-standing question in plant biology: why trees in North American and East Asia produce red leaves in autumn. Using a comparative geographical analysis, they present evidence supporting a new hypothesis: that red autumn leaves are a relict of adaptation to different climates and herbivores of the Tertiary.

Emerging themes and future directions

Adaptation and selection will remain central foci of research activity for as long as organisms interact with the physical and living components of their environments. Building on Darwin's foundation, evolutionary studies now aim to reveal the molecular and genomic properties of these interactions at the individual, population, community and phylogenetic levels. Recent work provides keen insights into the environmental context dependence of gene expression, the complex interplay of biotic and abiotic selection pressures in real habitats, the pathways and constraints to adaptive transitions, and the nature of evolutionary innovations in functional and reproductive traits. Evolutionary perspectives are generating new ways of thinking about plant development, physiology, geographic distribution, and community ecology. These interdisciplinary research efforts will also continue to inform invasion biology and biodiversity conservation in powerful ways.

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Editor

Acknowledgements

This special issue of *New Phytologist*, which is devoted to plant adaptation, celebrates the bicentennial of Charles Darwin's birth and the sesquicentennial of the publication of *On the origin of species*. This issue was edited by Richard Abbott, David Ackerly, Laura Galloway, Mark Rausher, Ruth Shaw and Sonia Sultan. In addition, Laura Galloway, Mark Rausher, Ruth Shaw and Holly Slater provided comments and suggestions on the introductory Editorial. We thank the many Authors and Reviewers who have also contributed to this special issue and we hope that you, our Readers, will enjoy and take inspiration from it.

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Key words: adaptive transition, evolutionary innovation, genetic constraint, natural selection, range expansion, selfing.

Letters

Specialization of flowers: is floral orientation an overlooked first step?

At the very inception of pollination biology, both floral symmetry and floral presentation received attention from a functional perspective (Sprengel, 1793). Sprengel suggested that floral symmetry (radial or bilateral) dictates insect movement in the flower, such that bilateral symmetry results in more directed and predictable insect movement. By contrast, floral presentation (where 'vertical' is facing upwards; 'horizontal' is roughly parallel to the ground; and 'pendant' is facing downwards; Fig. 1a–c, respectively), hereafter referred to as floral orientation, was thought to be more closely associated with protection from the elements and inflorescence architecture. Sprengel emphasized that nectar and pollen in horizontal and pendant flowers on spikes are protected from the rain, whereas flowers facing horizontally also provide a more visible display when packaged in a spike or raceme than if projected upwards (for example, Lamiaceae). Flower orientation as an adaptive response to abiotic factors has been documented, for example protection from rain (Tadey & Aizen, 2001; Huang *et al.*, 2002; Aizen, 2003; Ushimaru *et al.*, 2006; Sun *et al.*, 2008), and the regulation of heat load in the flower, as a consequence of either water conservation (Patiño *et al.*, 2002) or, in the case of alpine plants, as a pollinator reward (Hocking & Sharplin, 1965; Kevan, 1975; Totland, 1996) or enhanced environment for pollen germination (Galen & Stanton, 2003). However, how floral orientation might directly influence the approach of a pollinator to a flower has received little attention, despite the historical and contemporary interest in the latter in relation to floral symmetry.

The recognition that zygomorphy dictates pollinator movement within a flower, and therefore potentially pollination precision (Sprengel, 1793, Darwin, 1885), has led to considerable attention being devoted to the exploration of its functional and evolutionary significance (for example, Gomez *et al.*, 2006). Reviews have discussed the relationship of symmetry patterns with plant diversification (Donoghue *et al.*, 1998; Endress, 1999; Sargent, 2004) and plant–pollinator interactions (Stebbins, 1974; Giurfa *et al.*, 1999; Fenster *et al.*, 2004). Curiously, early workers recognized the potential adaptive significance of the horizontal orientation of flowers

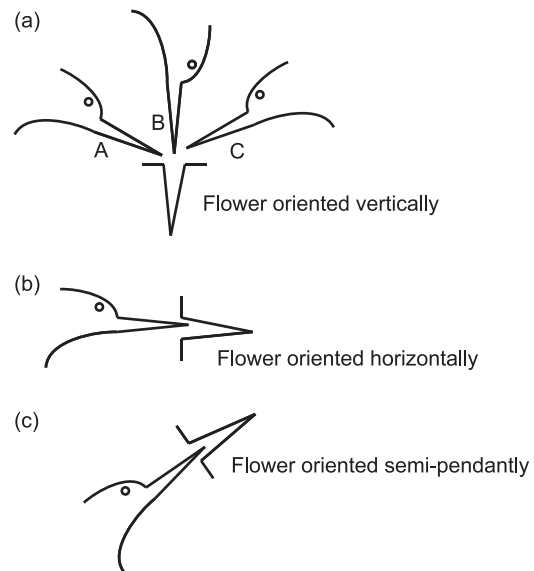


Fig. 1 (a) Approach angles to an upright, vertically facing *Silene virginica* flower. The hovering hummingbird can approach the corolla tube entrance or opening from one of two planes (and in between): either orthogonal to the tube opening (hummingbird is upright, positions A and C) or parallel to the tube opening (hummingbird is in a dive-bombing position, position B). Along each of these planes, the hummingbird can enter the flower with its forehead (point of contact with the anthers and stigma) from any direction (0° – 360°). Approach angles to a horizontal (b) or semi-pendant (c) *S. virginica* flower. The hovering hummingbird can approach the corolla tube from only one plane, parallel to the plane of opening of the tube. Along this plane, the hummingbird can enter the flower with its forehead from any direction (0° – 360°). However, only one orientation corresponds to the hummingbird entering the flower upright (arbitrarily designated the 90° angle), whereas the other angles reflect the entrance of the hummingbird either on its side (0° and 180°) or upside down (270°).

as a mechanism to ensure consistent pollinator directionality in approach to, and behaviour on, the flower. Robertson (1888a,b,c) recognized that a pollinator will approach an upward-facing flower from any direction, whereas a pollinator can approach a horizontally oriented flower essentially from one direction and in one position [the pollinator is upright, as pointed out by Sprengel (1793)]. Indeed, Ushimaru and Hyodo (2005) demonstrated that floral orientation can strongly influence pollinator directionality in the zygomorphic flowers of *Commelina communis*. Pollinators were more likely to contact the anthers and simultaneously touch the stigmas when *C. communis* was in its normal horizontal position than when the flowers were tethered vertically towards the sky.

This occurs because the pollinators approach the flower from many different directions when tethered upright, and thus the zygomorphic flowers only impose directionality of pollinator movement when facing horizontally, their natural position (see also Berg, 1960). Many plant species pollinated by hovering pollinators also present their flowers horizontally or in a pendant fashion, suggesting that this floral orientation may have adaptive significance in terms of pollination precision.

The question remains, however, whether floral orientation alone, independent of floral symmetry, influences pollinator directionality and the consistency of pollinator movement. Directionality of pollinator movement to and within a flower may ultimately contribute to a plant's overall reproductive success in terms of efficient export and receipt of pollen (Inouye *et al.*, 1994). This is because consistent directionality of the pollinator's movement allows the opportunity for the plant to respond evolutionarily by clustering its reproductive parts within the flower. This clustering may increase both the accuracy and precision of pollen transfer (*sensu* Armbruster *et al.*, 2004; Hansen *et al.*, 2006). Accuracy is increased by the better correspondence of pollen placement with the position of stigma contact with the pollinator, and vice versa. Clustering of stamens or stigmas results in contact with the pollinator in a smaller area and lowers the variance in pollen placement and stigma contact, hence increasing the precision of pollination. Because this is advantageous only if pollinators position themselves consistently as they enter or land on a flower, a more thorough understanding of the adaptive floral features that enforce consistent directionality in the approach of a pollinator to a flower is needed.

In this article, we investigate the role of floral orientation, vertical vs. horizontal vs. semi-pendant, in determining the direction of approach and orientation of a pollinator to radially symmetrical flowers. Hence, we investigate how floral orientation alone may contribute to more precise pollination by making the pollinator's approach and orientation to a flower more consistent. We measured the directionality of pollinator visits to artificial flowers (see Fenster *et al.*, 2006), made to resemble hummingbird-pollinated *Silene virginica* (Caryophyllaceae; Fenster and Dudash, 2001), in response to different floral orientations. We present evidence that radial or actinomorphic flowers that are presented horizontally promote consistent, restricted directionality of movement and positioning by hummingbirds compared with vertically presented flowers.

Materials and Methods

Silene virginica has radially symmetrical, horizontally oriented flowers that are red, scentless and tubular with relatively copious and dilute nectar (Reynolds *et al.*, 2009). They are almost exclusively pollinated by the ruby-throated hummingbird, *Archilochus colubris* (Fenster & Dudash, 2001; Reynolds *et al.*, 2009). We induced hummingbirds to

participate in a choice experiment by providing a hummingbird feeder in an open area surrounded by forest, and then removing the feeder and presenting artificial flowers in an array. The feeder was oriented close to the ground, similar to the plants in nature, somewhat analogously to flowers with their corolla tube openings oriented horizontally. The experiments were conducted at Mountain Lake Biological Station (37°22'32"N latitude, 80°31'20"W longitude). The construction of the artificial flowers has been described in Fenster *et al.* (2006). To ensure that nectar did not influence the choice of flowers, all artificial flowers were filled with 200 µl of 23% sugared water, *c.* 10–15 times the amount of nectar normally found in *S. virginica* flowers, but of the same sucrose concentration. To ensure that differences in nectar reward did not influence hummingbird approach, the experiment was halted and all artificial flowers were refilled at the very first sign that the nectar was depleted from any artificial flower in the array.

The experiment consisted of 12 artificial flowers arranged 0.4 m apart with three on each side of the perimeter of a square. The flowers, schematically illustrated in Fig. 1, were oriented vertically (floral tube of the flower facing the sky; Fig. 1a), horizontally (flower parallel to the ground; Fig. 1b) and semi-pendant (flower oriented *c.* 45° downward from the horizontal; Fig. 1c). The position of a flower within each side of the array was random. Thus, there were four replicates (one replicate per side of the array) for each floral orientation in a given array and trial. After each observation period or trial, the position of a particular flower orientation was re-randomized for each side of the array. The visitation patterns of hummingbirds were observed for four observation periods or trials of 20 min each in one afternoon in 2005, and for five afternoons, two 60-min observation periods per day, in 2007. In 2005, 10 hummingbird individuals were observed in the array at a single instance, and up to four hummingbirds were observed in a single instance in 2007. Thus, hummingbird approach to the artificial flowers represents the behaviour of at least 10–14 different ruby-throated hummingbirds across 2 yr. Ideally, hummingbirds should have been identified by individual, with each bird used as a replicate for testing the effect of flower orientation on bird approach direction. Consequently, our inference on how floral orientation affects all hummingbird approach decisions depends on our assumption that each visit represents a sample of behaviours that all hummingbirds would exhibit if presented with the different floral orientation treatments, perhaps resulting in an inflation of the Type I error rate.

Because pollinators generally prefer to remain upright throughout their visit, approaches are expected to be from any direction in the hemisphere above a vertically oriented flower, but most likely in any of the four compass directions on the plane perpendicular to the tube opening (Fig. 1a, A and C) (as opposed to 'dive-bombing' from above: Fig. 1a, B). The direction of visitation to the vertical flowers was quantified in the context of the arrangement of the array. Thus, a

hummingbird's approach to a vertical flower was quantified as 0° (south), 90° (east), 180° (north) and 270° (west), where each of the angles represents directionality independent of the position of the observer. If the hummingbird approached the flower at an intermediate angle, it was assigned to the closest major category of direction it represented. The assignment of directionality to only four cardinal directions makes our results conservative, as we have reduced the possible directionality options to only four. For horizontally or semi-pendantly oriented flowers, we noted the position of the hummingbird's forehead relative to the petal in the 12 o'clock or 90° position, again corresponding to an upright approach to the flower by the hummingbird (Fig. 1).

The extent of consistent directionality by the hummingbird visitor to a particular floral orientation was assessed by χ^2 analysis, where random orientation of the hummingbird was the expected frequency (all four approach angles equally used by the hummingbirds).

Results

Across the 2 yr of the study, a total of 471 hummingbird visits was observed and, of these, 224, 122 and 125 visits were to vertically oriented, horizontally oriented and semi-pendant flowers, respectively. The smaller numbers of observations to horizontally oriented and semi-pendant flowers was not a result of lower overall visitation rates to these treatments. Rather, because consistent directionality was observed (> 99% of all visits were in the same direction) for the horizontal and semi-pendant artificial flowers, observer attention was focused on the approach behaviour of the hummingbirds to the vertically oriented flowers, resulting in their greater sample size.

Of the 224 visits to vertically oriented flowers, all were on the plane perpendicular to the flower tube entrance (bird position portrayed in Fig. 1a, A and C). The visits came from all compass directions with 61, 51, 55 and 57 hummingbird visits approaching the flowers from the 0°, 90°, 180° and 270° angles, respectively. This represents a random directional approach to the upright flower by the hummingbirds ($\chi^2 = 0.2143$, d.f. = 3, $P = 0.9505$). As a consequence, the birds would have contacted fertile parts and corolla from any side. By contrast, all 122 visits to the horizontal flowers were by hummingbirds that approached the flower *en face* and hovered in a corresponding upright position, and 124 of 125 visits to the semi-pendant flowers were also *en face* and upright. These birds contacted the flowers in a consistent fashion and would have contacted the same side or part of the sexual parts and corolla if the flowers themselves were oriented consistently relative to vertical (as is nearly always the case in real flowers). In the one exceptional visit, the hummingbird approached the semi-pendant flower with its wings tilted towards the sky and ground. This exceptional approach would have resulted in contact with the sexual parts of the

flower at a different location on the head relative to the upright visits to the horizontal and semi-pendant flowers.

Discussion

Bilateral symmetry in flowers is commonly viewed as enhancing pollination accuracy and precision (Armbruster *et al.*, 2004) by 'forcing the pollinator to occupy a certain position' (Faegri & van der Pijl, 1979: p. 62; see also Darwin, 1885). This perspective is reflected in the observation that radially symmetrical flowers often have their anthers and stigmas diffusely distributed in the flower because pollinator approach to the flower can be from more than one direction (Neal & Anderson, 2005). In this article, we demonstrate that the pattern of floral symmetry is not the sole factor influencing pollinator approach. A simple change in flower orientation from vertical to horizontal can dramatically change pollinator approach and orientation relative to the floral parts from random to directional. The important consequence is that pollinator approach to a flower is now consistent and predictable. When a hummingbird approaches a vertical-facing flower, it can approach on a plane orthogonal to the tube entrance from any direction. By contrast, for a horizontal or semi-pendant flower, the hummingbird is prevented from approaching from the back and sides of the flower, and is oriented consistently in front of the flower by its predilection to remain upright (Warrick *et al.*, 2005; also evident from our personal observations of animals nearly always flying in an upright position). Consequently, the hummingbird approaches the flower in one direction, *en face*, or on the same plane as the flower tube opening, and always with its head in the 12 o'clock position. As a result, it will consistently contact certain floral parts, for example, the upper petals with its forehead and the lower petals with its chin. If the sexual parts were in the middle, they would probably contact the forehead consistently, as the bird usually approaches the flower from just slightly below the horizontal plane. Clearly, the next step is to verify that pollen transfer precision is increased by going from the vertical to the horizontal position.

If a pollinator's movement and orientation are consistent, natural selection should favour a corresponding shift in the position of the reproductive parts of the flower, such that more pollen is removed and deposited per visit by the pollinator, that is, increasing the accuracy of pollination (Armbruster *et al.*, 2004). Therefore, the reproductive parts should also evolve to contact the pollinator either on the dorsal surface (forehead) or the ventral surface (chin), in concert with an evolutionary transition from vertical- to horizontal-facing flowers. In fact, *S. virginica* exhibits radially symmetrical petals with zygomorphic placement of its reproductive parts, such that its stigmas are clustered in the centre of the floral tube and its anthers emerge at the 12 o'clock position as predicted. This arrangement is referred to as moderate zygomorphy (Neal *et al.*, 1998; Endress, 1999), and it has been hypothesized to

enhance the accuracy of pollen transfer, as anthers and/or stigmas can be clustered in parts of the flower that are consistently contacted by the pollinator (Vogel, 1996). Clustering of reproductive parts decreases the variance of placement, hence simultaneously increasing the precision and accuracy of pollination. We hypothesize that the consistent upright approach by hummingbirds to horizontally oriented flowers imposes selection on the clustering of reproductive parts within the flower. By contrast, vertical flowers need to place their anthers and stigmas diffusely throughout the flower or risk 'losing out' on approaches from some directions. For example, the closely related, completely radially symmetrical and vertically oriented flowers of *S. caroliniana* present their reproductive parts throughout the entire circumference of the vertical floral tube, as predicted. The comparison of these two closely related species prompts us to hypothesize that the orientation of flowers is indeed the first evolutionary step towards the evolution of zygomorphy. Horizontal orientation sets the selective stage for the evolution of slight zygomorphy in sexual parts, followed by the evolution of full zygomorphy. It should be possible to test this hypothesis with phylogenetic comparative approaches in species-rich groups that exhibit repeated origins of zygomorphy, for example Boraginaceae, Solanaceae and Lamiales (Reeves & Olmstead, 2003).

Large-bee-pollinated and horizontally oriented flowers are typically packaged in racemes or spikes (Sprengel, 1793), which impose much greater directionality of bee movement within the inflorescence relative to other floral arrangements, as bees typically approach a raceme or spike inflorescence from the bottom and subsequently forage upwards (Jordan & Harder, 2006). Thus, for large-bee-pollinated species with horizontal flowers arranged in a spike or raceme, consistent directionality of pollinator movement can be expected at both the flower and inflorescence level. We note that an inflorescence architecture that facilitates movement by walking or crawling between flowers would diminish the constancy of pollinator approach.

Downward-facing or semi-pendant artificial flowers also impart strong directionality to pollinator movement. However, downward-facing flowers are thought to be at a disadvantage, because they are less easily seen by pollinators (Sprengel, 1793). There is limited but intriguing evidence from both insects and birds of reduced visitation rates to downward-facing flowers compared with vertically oriented flowers, as expected (Fulton & Hodges, 1999; Giurfa *et al.*, 1999; Ushimaru & Hyodo, 2005; Ushimaru *et al.*, 2006). If upward-facing flowers are more likely to be seen by pollinators (at least in flat habitats), increased pollen transfer accuracy and precision associated with imposed pollinator directionality may offset the attractiveness disadvantage.

Although the results of this study were anticipated by earlier workers (Sprengel, 1793; Robertson, 1888a,b,c), we are not aware of any experimental work that has confirmed our intuition of how flower orientation can consistently direct

pollinator movement independent of floral symmetry. Clearly, more attention should be focused on floral orientation and its role in the enhancement of plant fitness through directing pollinator visitation behaviour to a flower. The manipulation of floral orientation effects on pollinator directionality should be tested across a wide range of floral morphologies, beyond the tubular flowers tested here, and for other pollinators, including hovering and nonhovering visitors.

Acknowledgements

The authors are grateful to R. Reynolds and C. Williams for help in the initial stages of this project, E. Nagy, H. Wilbur and E. Brodie for logistical support and encouragement, P. Endress, J. Hereford, S. Marten-Rodriguez, M. Rausher, R. Reynolds H. Wilbur and two anonymous reviewers for comments on previous versions of the manuscript, and funding from NSF DEB 0108285 to C. Fenster and M. Dudash.

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Why red-dominated autumn leaves in America and yellow-dominated autumn leaves in Northern Europe?

In North America and East Asia, the autumn is dominated by many tree species with red autumn leaves (e.g. Hoch *et al.*, 2001; Lee *et al.*, 2003). We examined the distribution of each of the 290 tree species with red autumn leaves listed in Archetti (2009a) and found that most of them also grow in North America and East Asia. By contrast, yellow autumn leaves dominate the parallel phenomenon of autumn leaf colouration in Finland and most of temperate Northern Europe (Holopainen & Peltonen, 2002). While for many years these colours were considered a by-product of chlorophyll degradation (chlorophyll ceases to mask the colours) it is clear that this is not the case, at least for red autumn leaves that produce anthocyanins shortly before leaf shedding (Sanger, 1971; Matile, 2000; Hoch *et al.*, 2001; Lee, 2002; Lee & Gould, 2002). The proportion of tree species with red autumn leaves in the flora and landscape of Northern Europe is small; for example, four indigenous tree species (*Prunus padus*, *Prunus spinosa*, *Sorbus aucuparia* and *Acer platanoides*) reaching their northernmost distribution in Northern Europe (Alanko, 2001; Holopainen & Peltonen, 2002), and only 24 such species in the whole of Europe (according to our examination of the distribution of tree species with red autumn leaves listed in Archetti, 2009a), while in eastern North America and East Asia both the proportion in the landscape and the actual number of tree species with red autumn leaves are much greater; at least 89 species in a subset of the woody flora of North America (e.g. Lee *et al.*, 2003; and examination of the distribution of the 290 tree species with red autumn leaves listed in Archetti, 2009a) and at least 152 species in East Asia. The question of red-dominated autumn leaves received much research attention in the last decade: we found more

than 80 papers discussing it. Archetti (2009a) has already described red autumn colouration in 290 temperate tree species belonging to 70 genera, and stated that the full list is probably even longer. Because the physiological, ecological and evolutionary aspects of the hypotheses were discussed in great detail in those numerous studies we will mention them only briefly. These hypotheses are of three types: physiologically oriented, anti-herbivory oriented, and a combination of the two. Recently the topic of the evolution of red autumn colouration as an anti-herbivory defence has been hotly debated, dividing physiologists and ecologists, who generally ignored each other and proposed only physiological or herbivory-related explanations, respectively. At the same time, ecologists have not agreed upon the various anti-herbivory functions of autumn colouration. While the simultaneous physiological and defensive roles of red autumn colouration were recently recognized by many (e.g. Lev-Yadun & Gould, 2007; Archetti *et al.*, 2009), the difficulties in proving some of the anti-herbivory functions remain.

In general, scavenging of reactive oxygen species and defence from photoinhibition under low temperatures (e.g. Matile, 2000; Hoch *et al.*, 2001, 2003; Lee, 2002; Lee & Gould, 2002; Wilkinson *et al.*, 2002; Schaberg *et al.*, 2003, 2008; Ougham *et al.*, 2005; Lev-Yadun & Gould, 2007, 2008) are the main physiological functions of red autumn colouration, but there is also a relationship to reproductive effort (Sinkkonen, 2006a,b) or branch die-back (Sinkkonen, 2008). One of the major anti-herbivory hypotheses is the coevolutionary hypothesis (e.g. Archetti, 2000; Hamilton & Brown, 2001; Archetti & Brown, 2004). When the coevolutionary hypothesis was proposed it caused great debate for a variety of reasons, including limited support from recent field and laboratory data concerning aphid biology and aphid–tree relationships (e.g. Holopainen & Peltonen, 2002; Wilkinson *et al.*, 2002; Schaefer & Wilkinson, 2004; Schaefer & Rolshausen, 2006, 2007). Other anti-herbivory hypotheses are: undermining camouflage (Lev-Yadun *et al.*, 2004; Lev-Yadun, 2006, 2009; Lev-Yadun & Gould, 2007, 2008), signalling that the leaves are going to be shed soon (Lev-Yadun & Gould, 2007), aposematic colouration (Lev-Yadun & Gould, 2007, 2008; Archetti, 2009b; Archetti *et al.*, 2009; Lev-Yadun, 2009) and olfactory signalling (Holopainen, 2008). For the full list of the possible functions of red autumn leaf colouration see Archetti *et al.* (2009) and citations therein. In several papers discussing the significance of autumn colouration, an effort was made to integrate physiology and defence (e.g. Wilkinson *et al.*, 2002; Gould, 2004; Lev-Yadun *et al.*, 2004; Manetas, 2006; Lev-Yadun & Gould, 2007, 2008; Ougham *et al.*, 2008; Archetti *et al.*, 2009), or to focus on animal sensory aspects (e.g. Chittka & Döring, 2007; Holopainen, 2008; Döring *et al.*, 2009). While all these detailed discussions focused on the biological origin or physiological and ecological functions of autumn colouration, we focus on an unexplored aspect of its origin in time in relation to past global climatic changes.

Here we use the prevalence of red autumn colouration of trees in North America (Lee *et al.*, 2003) and East Asia (our examination of the distribution of the 290 tree species with red autumn leaves listed in Archetti, 2009a) versus the prevalence of yellow autumn leaves in Northern Europe (Holopainen & Peltonen, 2002; J. K. Holopainen & S. Lev-Yadun, field notes), along with known patterns of migration and extinction during the drastic climatic changes in the Tertiary and the Pleistocene (e.g. Milne & Abbott, 2002), as the basis for a new hypothesis. We propose that higher extinction rates of both trees and their insect herbivores in Europe as opposed to North America and East Asia seem to explain the difference, indicating that red autumn leaves are probably a relict Tertiary adaptation of temperate floras to past climates and herbivore faunas.

Discussion

The origin of the current temperate tree flora

The current land biota, with sharp differences between the adaptations to warm tropical and subtropical regions and cold temperate and arctic regions, is a relatively new phenomenon in geological and evolutionary time scales. After a very long warm period, with much lower thermal gradients between the tropical and polar regions that we are familiar with today (see Axelrod, 1966; Tiffney, 1985; Graham, 1993; Manchester, 1999), phases of cooling and glaciation alternating with warmer phases began in the mid Tertiary *c.* 35 million years ago, a process that culminated in the Pleistocene (Imbrie & Palmer-Imbrie, 1979; Tiffney, 1985; Zachos *et al.*, 2001). Such dramatic climatic changes selected for various physiological adaptations, including adaptations to cold environments (e.g. Axelrod, 1966; Stebbins, 1974; Tiffney, 1985; Takhtajan, 1991; Delcourt & Delcourt, 1993; Graham, 1993; Wen, 1999).

Several times during the Pleistocene, large areas in Europe and North America were covered by ice and could not support trees or any plants at all (Imbrie & Palmer-Imbrie, 1979; Tiffney, 1985; Graham, 1993; Wen, 1999; Hewitt, 2000; Milne & Abbott, 2002). During the cold phases, trees and many other organisms survived in warmer southern regions, termed refugia (e.g. Bennett *et al.*, 1991; Delcourt & Delcourt, 1993; Comes & Kadereit, 1998; Hewitt, 2000; Milne & Abbott, 2002). Some refugia were relatively northern and inland (e.g. the northern Balkans) where they were exposed to very low winter temperatures that should have had a stronger influence on herbivore extinction, and some relatively southern (e.g. the Iberian Peninsula and southern Italy) (Bennett *et al.*, 1991; Willis & van Andel, 2004; Provan & Bennett, 2008).

In Europe, northern refugia for broad-leaf trees during glaciations are known for a small number of deciduous species, none of which has red autumn leaves: *Alnus glutinosa* (green autumn leaves), *Betula pendula* (yellow), *Fagus sylvatica* (yellow),

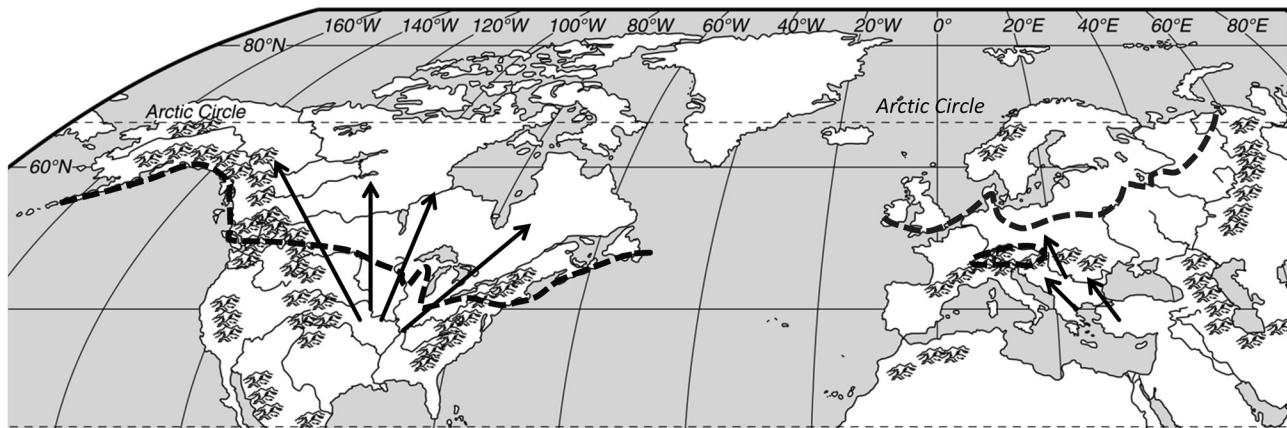


Fig. 1 Schematic representation of migration routes from southern refugia of woody plants after the retreat of the waves of glaciations during the Pleistocene in North America and Europe (arrows). The southern limit of the last glaciation is marked by a dashed line.

Fraxinus excelsior (green and sometimes yellow), *Salix* sp. (yellow), *Corylus avellana* (yellow) and *Frangula alnus* (yellow) (Bhagwat & Willis, 2008).

Mountain ridge direction and severity of ice-age extinctions

In eastern North America especially, but also in western North America and East Asia, the direction of the mountain ridges is from north to south (Tiffney, 1985; Hewitt, 2000; Milne & Abbott, 2002; Soltis *et al.*, 2006). By contrast, in Europe, the Alps form an east–west ridge (Tiffney, 1985; Milne & Abbott, 2002; Milne, 2004; Soltis *et al.*, 2006) (Fig. 1). Accordingly, in North America and East Asia, when the southward-advancing ice damaged the biota, tree species and their specific insect herbivores could migrate to the warmer south in the valleys among the mountains, or along the ridges, and vice versa during the retreat of the ice, resulting in the preservation of many ancient floral and faunal elements. In Europe, during the repeated drastic climatic changes of the Pleistocene, the biota was trapped between the advancing ice from the north on the one hand and ice from the Alps in the south on the other (Imbrie & Palmer-Imbrie, 1979), and a larger proportion of the species became extinct, leaving a smaller number of species that spread from several refugia during warmer periods (Tiffney, 1985; Comes & Kadereit, 1998; Milne & Abbott, 2002; Milne, 2004; Soltis *et al.*, 2006). The great differences in extinction between Europe and other continents can be seen in the much smaller number of North European deciduous tree species (Milne & Abbott, 2002) compared with eastern North America and East Asia (e.g. Milne & Abbott, 2002; Lee *et al.*, 2003; the results of our examination of the distribution of the 290 tree species with red autumn leaves listed in Archetti, 2009a). Many more Tertiary elements are therefore found in North America and East Asia than in Northern Europe (Tiffney, 1985; Milne & Abbott, 2002).

Anachronisms in plant adaptations as evidence of extinct faunas

There are several independent sets of evidence for anachronistic adaptations in plants to extinct faunas. The first is the nature of various tropical fruits that are adapted to large mammalian frugivores (Janzen & Martin, 1982; Barlow, 2000; Guimarães *et al.*, 2008). A second adaptation is the very spiny cacti that were proposed to reflect the extinct megafauna of North America (Janzen, 1986) and other defended North American plant taxa (White, 1988; Barlow, 2000). A similar phenomenon of spiny plants that reflect extinct large grazers such as auroches and tarpans was also proposed for northwestern Europe (Bakker *et al.*, 2004). A third proposed anachronistic adaptation is of divaricate branching in New Zealand trees and shrubs as a defence against the extinct moas (Greenwood & Atkinson, 1977; Diamond, 1990; Bond *et al.*, 2004), and in similar plants in Madagascar as defence against the extinct elephant birds (Bond & Silander, 2007). In all these cases, the plants may currently use the anachronistic adaptations as functional solutions in a different biological or environmental setting (Janzen & Martin, 1982; Janzen, 1986; Barlow, 2000; Howell *et al.*, 2002; Guimarães *et al.*, 2008). There is no reason to assume that the cited cases of botanical anachronisms are the only ones, as will be discussed in the conclusions.

While the examples we give of fossil plant adaptations to extinct faunas are from large vertebrates, there is no reason to assume that the same is not true for plant–insect interactions. The fact that fossil insects are less extensively studied, and that it is harder to find a specific connection between a fossil insect and its host plant, does not rule out the probability that many insect species probably became extinct during the Pleistocene. There are solid experimental and field data concerning the sensitivity of aphid and other herbivorous insect eggs and all their life stages to very low temperatures (Niemelä, 1979; Tenow & Nilssen, 1990; Strathdee *et al.*, 1995; Strathdee &

Bale, 1998). This sensitivity must have resulted in extinction of many insect species during the drastic climatic changes of the Pleistocene, leaving defaunated floras in all continents, but especially in Northern Europe, similar to the defaunated cacti in North America (e.g. Janzen, 1986).

Red leaves in trees

Red colouration in leaves of woody plants is common in three major situations. The first is the young red leaves that are common in the tropics (Juniper, 1994; Richards, 1996; Dominy *et al.*, 2002; Lee, 2007) as well as in subtropical regions (Karageorgou & Manetas, 2006). The second is nonsenescing leaves of both deciduous and evergreen species that turn red under various physiological stresses, especially those associated with low temperatures (Chalker-Scott, 1999; Matile, 2000; Feild *et al.*, 2001; Hoch *et al.*, 2001, 2003; Lee, 2002; Lee & Gould, 2002; Close & Beadle, 2003; Gould, 2004; Hughes & Smith, 2007; Ougham *et al.*, 2005). The third is red autumn leaves (e.g. Matile, 2000; Archetti, 2000; Hamilton & Brown, 2001; Hoch *et al.*, 2001; Lee, 2002).

Boreal shrubs with red autumn leaves

Further support for our hypothesis of an ancient Tertiary origin of red autumn colouration stems from the fact that dwarf shrubs with red autumn leaves, rather than trees, dominate the northern territories of Scandinavia. For instance, the deciduous species *Arctostaphylos alpina* with its circumpolar distribution (Hämet-Ahti *et al.*, 1992) is one of the most common dwarf shrub species in mountainous areas of Lapland, and has bright red autumn leaves. In lowlands and forested areas, the deciduous shrubs *Vaccinium myrtillus* and *Vaccinium uliginosum* have reddish or darker brown autumn colouration. Several evergreen dwarf arctic shrub species of Northern Europe or Alaska (*Andromeda polifolia*, *Cassiope tetragona*, *Diapensia lapponicum*, *Dryas integrifolia*, *Empetrum nigrum*, *Ledum palustre*, *Oxycoccus microcarpus*, *Pyrola grandiflora*, *Rhododendron lapponicum* and *Vaccinium vitis-idaea*; Oberbauer & Starr, 2002) have red winter and spring leaves. The autumn landscape in the treeless far northern parts of both Scandinavia and Alaska is conspicuously dominated by red leaf colouration expressed only by very low shrubs.

There is a critical difference in the sensitivity of trees and shrubs to extinction when drastic climatic changes such as glaciation occur (e.g. Milne & Abbott, 2002). Trees are much larger and have a much longer life span and generation time than shrubs, which makes trees at an individual and evolutionary level less flexible and more susceptible to extinction. By contrast, the boreal shrubs we discuss not only have much smaller size and a shorter generation time, but usually have berries so that their seeds can be dispersed over large distances by animals. Shrubs can also manage much better than trees in colder and less productive habitats because their low stature allows them to enjoy an isolating snow cover in winter. Shrubs thus could find more

refugia than trees in periods of glaciation. All these differences allowed shrubs with red autumn leaves to escape extinction where trees could not survive. Moreover, if red autumn leaves are at least partially an anti-herbivory adaptation (e.g. Archetti, 2000; Hamilton & Brown, 2001; Archetti & Brown, 2004; Lev-Yadun *et al.*, 2004; Manetas, 2006; Lev-Yadun & Gould, 2007; Archetti *et al.*, 2009), the persistence of shrubs during periods of glaciation in the Pleistocene also allowed their herbivores to find refuge from extinction. The refuge of their specific herbivores is related to the ability not only to feed, but also to be sheltered from extreme low temperatures under the snow cover, like their hosts, continuing their role in selection for red autumn leaves.

The proposed historical origin (in geological era perspective) of red autumn colouration

We propose that, as temperate deciduous trees are of ancient (Cretaceous or Tertiary) tropical or subtropical origin (Axelrod, 1966; Stebbins, 1974; Tiffney, 1985; Milne & Abbott, 2002), it is possible to reconstruct a probable evolutionary route from young (e.g. Richards, 1996; Lee & Collins, 2001; Lee, 2007) and senescing (Lee & Collins, 2001) red leaves in tropical trees, through autumn- and winter-red leaves of evergreens (e.g. Chalker-Scott, 2002; Hughes & Smith, 2007), to red autumn leaves (e.g. Matile, 2000; Hoch *et al.*, 2001; Lee *et al.*, 2003) in trees that acquired the deciduous habit. The fact that, out of 399 tropical tree species studied, some 13.5% expressed anthocyanin during senescence (Lee & Collins, 2001), a ratio similar to the 12.2% of species with red autumn leaves found by Archetti (2009a) in his broad taxonomic review of current temperate floras, also supports the ancient origin of red autumn leaves.

A broad phylogenetic analysis of the origin of red autumn colouration in 2368 tree species indicated that this character evolved independently in temperate trees at least 25 times (Archetti, 2009a). There are several possible periods when red autumn leaves of deciduous trees could have evolved. First, it could be an ancient, Tertiary adaptation that was selected for during the periods of global cooling that began in the mid-Tertiary. A second period that could have strongly selected for such an adaptation is the Pleistocene, which started approximately 2.6 MY ago and exhibited dramatic and repeated climatic changes. Finally, it may be a recent Holocene (the end of the Pleistocene, which occurred some 11 000 years ago) adaptation. A combination of some of these is also possible. The question is which of these scenarios is most likely. The repeated evolution of the red autumn leaf colour in many tree taxa that have a long generation time and therefore slow evolution is a good indication of an ancient origin. We believe that the conspicuous differences in the distribution of red autumn colouration in eastern North America and East Asia (where many taxa have red autumn leaves) and Northern Europe (which is poor in red autumn colouration) are the key to solving this puzzle. If adaptations for low temperatures *per*

se were the selective agent for red leaf colouration, we would expect that the Scandinavian autumn would have been as red as the North American or East Asian autumn, but it is yellow. Alternatively, yellow autumn leaves would have dominated the autumn landscape of all continents. So, while we agree that anthocyanins provide several physiological solutions under low temperatures, as proposed previously (e.g. Matile, 2000; Hoch *et al.*, 2001, 2003; Lee, 2002; Lee & Gould, 2002; Wilkinson *et al.*, 2002; Schaberg *et al.*, 2003, 2008; Ougham *et al.*, 2005; Lev-Yadun & Gould, 2007; Archetti *et al.*, 2009), there is clearly no inherited physiological problem in functioning successfully with yellow autumn leaves under similar low autumn temperatures, as seen in *Betula* sp., *Populus* sp. and *Salix* sp. and the majority of deciduous temperate tree taxa (e.g. Archetti, 2009a). The possibility that the trees with yellow autumn leaves cannot produce anthocyanins should be dismissed because many temperate taxa with yellow autumn leaves have red pigmentation in various parts of their canopy, for example during spells of cold weather during leaf flush at the beginning of the growing season, or in their reproductive organs.

Conclusions

We propose that the solution to the problem of the origin of red autumn leaves in general, and their limited distribution in Northern Europe in particular, reflects the well-known difference in the extinction histories of trees in eastern North America and East Asia (lower extinction rate) and those in Northern Europe (higher extinction rate). If red autumn leaf colouration is the result of various ancient Tertiary adaptations of temperate floras of tropical and subtropical origins, then, while physiological adaptations are habitat and climate dependent and a geographical shift is enough to allow trees to grow when the climate changes, anti-herbivory adaptations may reflect not only the current herbivore fauna and their predators and parasites, but also many extinct animal species (both vertebrates and insects). As discussed above, anachronistic adaptations of plants to past faunas are well known. Herrera (1985), when discussing the lack of fine-scale adjustments in the coevolution of woody plants and animal seed dispersers, proposed that slower species turnover of woody plants over geological time compared with vertebrate dispersers may have favoured a sort of very diffuse coevolution. We propose a parallel scenario for red autumn leaves and herbivores (vertebrates and insects). Not only did trees with red leaves mainly become extinct in Europe, but when many of their herbivores became extinct, the driving selective agents for red autumn colouration also declined.

Thus, the anti-herbivory component of the character of red autumn leaves partly reflects anachronistic adaptations to past faunas, many of which became extinct. This will significantly reduce the chances of obtaining good theoretical and experimental evidence supporting anti-herbivory hypotheses using the current biota. Our hypothesis may calm the hot debate concerning autumn leaf colouration and coevolutionary hypotheses.

Acknowledgements

We thank Marco Archetti and two anonymous reviewers for their constructive comments.

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Key words: autumn leaf colour, coevolution, defence from herbivory, evolution, extinction, fossil adaptation, Pleistocene, Tertiary.



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