Phytologist

Research

Different rates of defense evolution and niche preferences in clonal and nonclonal milkweeds (*Asclepias* spp.)

Loïc Pellissier[1,2]*, Glenn Litsios[3,4]*, Mark Fishbein[5], Nicolas Salamin[3,4], Anurag A. Agrawal[6] and Sergio Rasmann[7]

[1]Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland; [2]Landscape Ecology, Institute of Terrestrial Ecosystems, ETH Zürich, Zürich, Switzerland; [3]Department of Ecology and Evolution, University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland; [4]Swiss Institute of Bioinformatics, Génopode Building, 1015 Lausanne, Switzerland; [5]Department of Botany, Oklahoma State University, Stillwater, OK 74078-3013, USA; [6]Department of Ecology and Evolutionary Biology, and Department of Entomology, Cornell University, 215 Tower Road, Ithaca, NY 14853, USA; [7]Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

Summary

• Given the dual role of many plant traits to tolerate both herbivore attack and abiotic stress, the climatic niche of a species should be integrated into the study of plant defense strategies.
• Here we investigate the impact of plant reproductive strategy and components of species' climatic niche on the rate of chemical defense evolution in the milkweeds using a common garden experiment of 49 species.
• We found that across *Asclepias* species, clonal reproduction repeatedly evolved in lower temperature conditions, in species generally producing low concentrations of a toxic defense (cardenolides). Additionally, we found that rates of cardenolide evolution were lower for clonal than for nonclonal species.
• We thus conclude that because the clonal strategy is based on survival, long generation times, and is associated with tolerance of herbivory, it may be an alternative to toxicity in colder ecosystems. Taken together, these results indicate that the rate of chemical defense evolution is influenced by the intersection of life-history strategy and climatic niches into which plants radiate.

Introduction

Herbivores consume up to one-fifth of the total primary production of plants (Marquis, 1984; Strong *et al.*, 1984), and to resist herbivory, plants have evolved a wide variety of defense traits, including toxic secondary metabolites that reduce plant palatability and tissue consumption (Schoonhoven *et al.*, 2005). The benefit of investment in defense, however, depends on the overall abiotic or biotic context of plant growth, such as resource availability, climatic conditions, abundance of herbivores, and the degree of food plant specialization in herbivores (Coley *et al.*, 1985; Strauss *et al.*, 2002; Lankau, 2007). Along with this recognition, relatively few broad-scale comparative studies have tackled the understanding of plant defense evolution in the context of environmental niches. Although across-clade studies have found support for the impact of environment on defense evolution (Endara & Coley, 2011), only within-clade studies can identify specific evolutionary patterns. For example, Pearse & Hipp (2012) showed that oak leaf defense production follows clines of temperature and humidity gradients, in which higher defenses are associated with regions of low temperature seasonality, mild

winters, and low minimum precipitation. Similarly, Moreira *et al.* (2014) found that patterns of pine defense induction are associated with temperature clines, in which species from warmer climates invested more in inducible defenses, and less in constitutive defenses. [Correction added after online publication 17 September 2015: 'colder' was changed to 'warmer' in the preceding sentence.]

Considering the environmental niche of species as a driver of plant defense strategies is further justified by the dual role of many plant traits to tolerate biotic as well as abiotic stresses (Rasmann *et al.*, 2014). Indeed, regrowth after damage is probably a generalized plant response to many types of tissue injury, including fire, trampling, frost, and herbivory (Belsky *et al.*, 1993). Specifically, clonality, as an extension of perenniality, appears to have been favored by highly disturbed and more unstable environments, including alpine areas (de Witte & Stoecklin, 2010), fire-prone ecosystems (Bond & Midgley, 2001; Litsios *et al.*, 2014), or anthropogenically disturbed habitats (Fahrig *et al.*, 1994). In this context, clonal growth represents an important alternative means by which plants can propagate themselves from carbon storage organs after strong perturbations (Fischer & Stocklin, 1997), including herbivory (Schmid *et al.*, 1988b; Pennings & Callaway, 2000), or under prolonged stress (de Witte

*Shared first authorship.

et al., 2012). As a consequence, and following strategic resource allocation theory (van der Meijden *et al.*, 1988; de Jong & van der Meijden, 2000), a tradeoff between clonal ability and chemical defense investment has been predicted (Agrawal *et al.*, 2015).

Across the globe, tropical areas have greater herbivore diversity than temperate ones (Schemske *et al.*, 2009), and warm tropical climate is tightly correlated with high insect species richness (Currano *et al.*, 2010). Therefore, it has been classically postulated that plants at higher latitudes or elevations experience lower herbivore pressure and should invest less in defenses against herbivores (Coley & Aide, 1991; Pennings *et al.*, 2009; Schemske, 2009; Pellissier *et al.*, 2012, 2014). Accordingly, tropical plants have been shown to produce more toxic alkaloids (Levin & York, 1978), latex (Lewinsohn, 1991), and cardenolides (Rasmann & Agrawal, 2011) than their temperate relatives. They also have tougher leaves of lower nutritional quality than temperate species (Coley & Aide, 1991). Additionally, arctic and alpine plants show reduced amounts of secondary metabolite production (Pellissier *et al.*, 2014) and resistance to herbivores (Pellissier *et al.*, 2012) than their tropical counterparts, although counter-examples exist (Moles *et al.*, 2011).

Therefore, if clonal species are favored in more unstable and unpredictable abiotic conditions, where herbivore pressure is reduced, plants should decrease their chemical defense arsenal, but invest more in their resprouting abilities, such as the development of underground vegetative mode of reproduction (Schmid *et al.*, 1988a; Stowe *et al.*, 2000; Wise & Abrahamson, 2005). Here we investigate the relationship between clonal reproduction, chemical defense investment, and plant distribution along temperature and precipitation gradients within milkweed species in the genus *Asclepias* (Apocynaceae). Chemical defense in milkweeds has been well studied and played a major role in the development of theories about plant–herbivore interactions (Brower *et al.*, 1972; Malcolm, 1995; Zalucki *et al.*, 2001; Agrawal & Fishbein, 2008; Agrawal *et al.*, 2009a; Mooney *et al.*, 2010; Dobler *et al.*, 2012). Milkweeds contain bitter-tasting steroids known as cardiac glycosides (or cardenolides) that gain toxicity by disrupting the sodium and potassium flux in animal cells (Malcolm, 1991; Agrawal *et al.*, 2012). In addition to being a well-studied system, *Asclepias* spp. are spread across a tremendous habitat and climatic range in the New World, and contain both nonclonal and clonal species (Woodson, 1954; Rasmann & Agrawal, 2011).

We tested the following hypotheses. Clonal species should occupy more unstable and unpredictable abiotic conditions. Given the expected decrease in herbivore pressure in colder conditions, plant investment in chemical defense should be lower in species living in temperate zones. If clonality allows increased tolerance of herbivory, and because tolerance and defense are generally negatively correlated, we expect a tradeoff between clonality and chemical defenses, assuming those strategies are redundant. Because clonal and nonclonal lineages may represent alternative strategies associated with different generation times, climatic environments, and herbivore pressure, different rates of defense evolution are predicted among the two life-history strategies.

Materials and Methods

Plant traits measurement

Seeds of 49 *Asclepias* species (six to 12 plants per species; see list of species names in Supporting Information Table S1) were collected by the authors, their colleagues, or purchased from native nurseries (Rasmann & Agrawal, 2011), and grown in a growth chamber (14 : 10 h, 24 : 18°C, day : night, 60% relative humidity) in potting soil (Metro-Mix Sun Gro Horticulture Canada CM Ltd, Vancouver, British Columbia, Canada). Plants were watered *ad libitum* and fertilized (N : P : K, 21 : 5 : 20, 150 ppm N (w/w)) once every week (for details, see Agrawal *et al.*, 2009a; Rasmann & Agrawal, 2011). *Asclepias* spp. do grow in various conditions, and in focusing on a common environment, our goal was to control for strong environmental (plastic) influences on the phenotype. All species grew well (none were etiolated, yellow, or otherwise appearing sick). To test for constitutive concentrations of foliar cardenolides and their inducibility (which is calculated as the difference between mean species values of induced and constitutive cardenolides) after 30 d of growth, leaves of *c.* 50% of the plants (three to six per species) were exposed to one first-instar monarch butterfly caterpillar (*Danaus plexippus*), a species that feeds almost exclusively on *Asclepias* species. The other plants remained undamaged. At 3 d after cessation of the herbivory treatment (*c.* 5% leaf damage per plant), all plants were harvested for cardenolide analysis of leaves, as previously described in Rasmann & Agrawal (2011).

In addition to our previous work (Agrawal *et al.*, 2015), we here further recorded two plant traits related to resprouting ability and clonality. First, we measured the number of buds on the rhizomes or caudices of each plant and weighed the rhizome or caudex biomass as quantitative assessments of resprouting abilities for each species (Table S1). We acknowledge that the root buds might be affected by different soil conditions, but by removing soil heterogeneity or other edaphic stresses we specifically aimed here to measure potential root bud production across all species. As nearly all *Asclepias* species produce new aerial stems from subterranean or superficial buds, we additionally characterized each species as clonal or nonclonal, based on the presence of long rhizomes that have the potential to generate physiologically independent ramets (Table S1). This characterization was based on the authors' observations in the field and in common garden experiments (M. Fishbein & A. A. Agrawal, pers. obv.; Fig. 1), and indeed reflects the abundance of root buds scored during the glasshouse experiment (Fig. 2). In addition, we measured phylogenetic signal for the clonality trait using the phylo.d function in R (Orme *et al.*, 2013), and found trait lability ($D = 0.97$, *P*-value testing whether D is significantly different from zero, i.e. phylogenetically conserved trait = 0.03).

Comparison of trait values

We collected occurrence data from GBIF (http://www.gbif.org) for each of the 49 species tested to build the temperature and precipitation components of their climatic niche, considering only
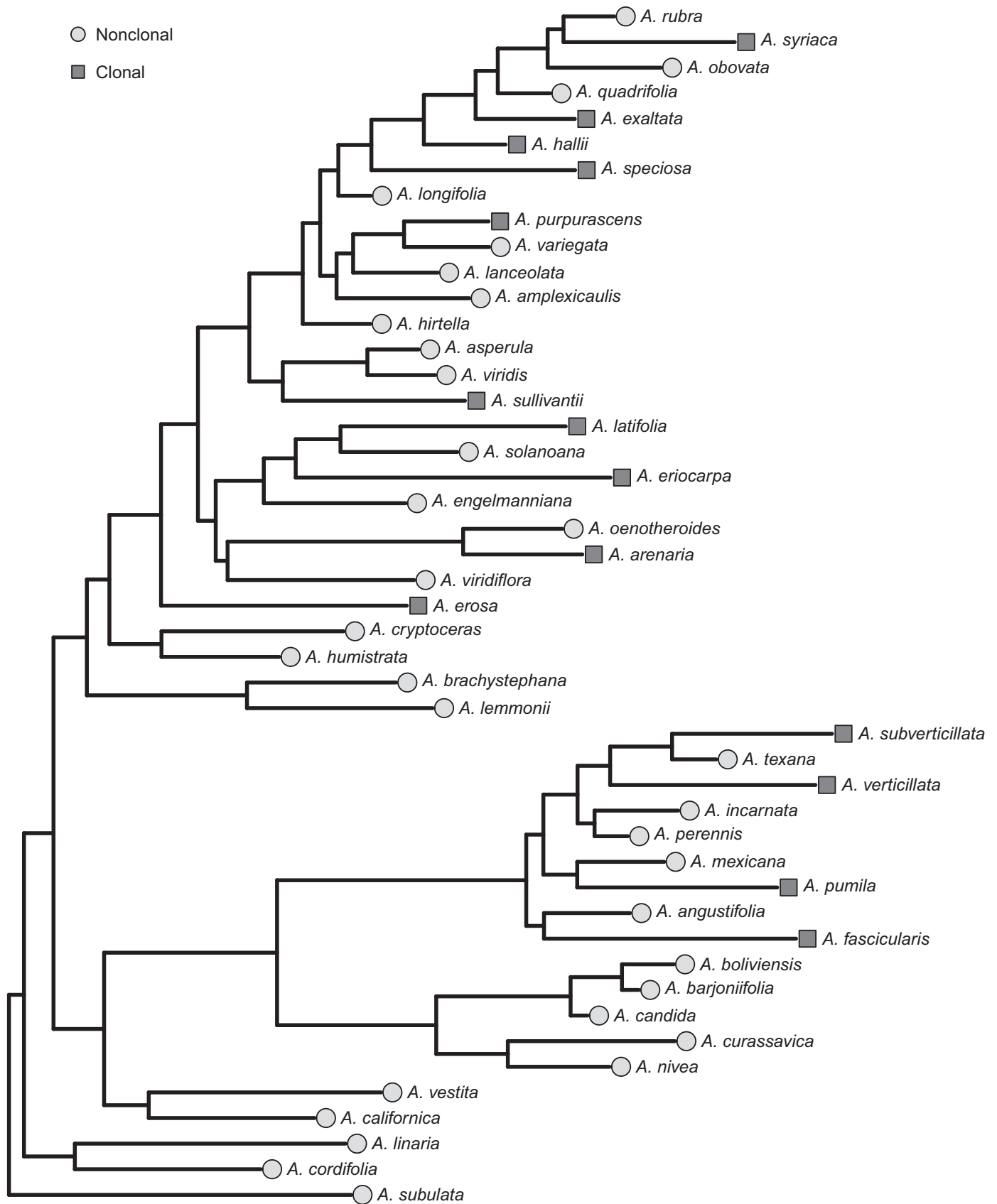
Fig. 1 Clonality (i.e. presence of long rhizomes) mapped on the pruned phylogeny of *Asclepias* (see Fishbein *et al.*, 2011) with clonal species coded as dark squares and nonclonal species as light circles.
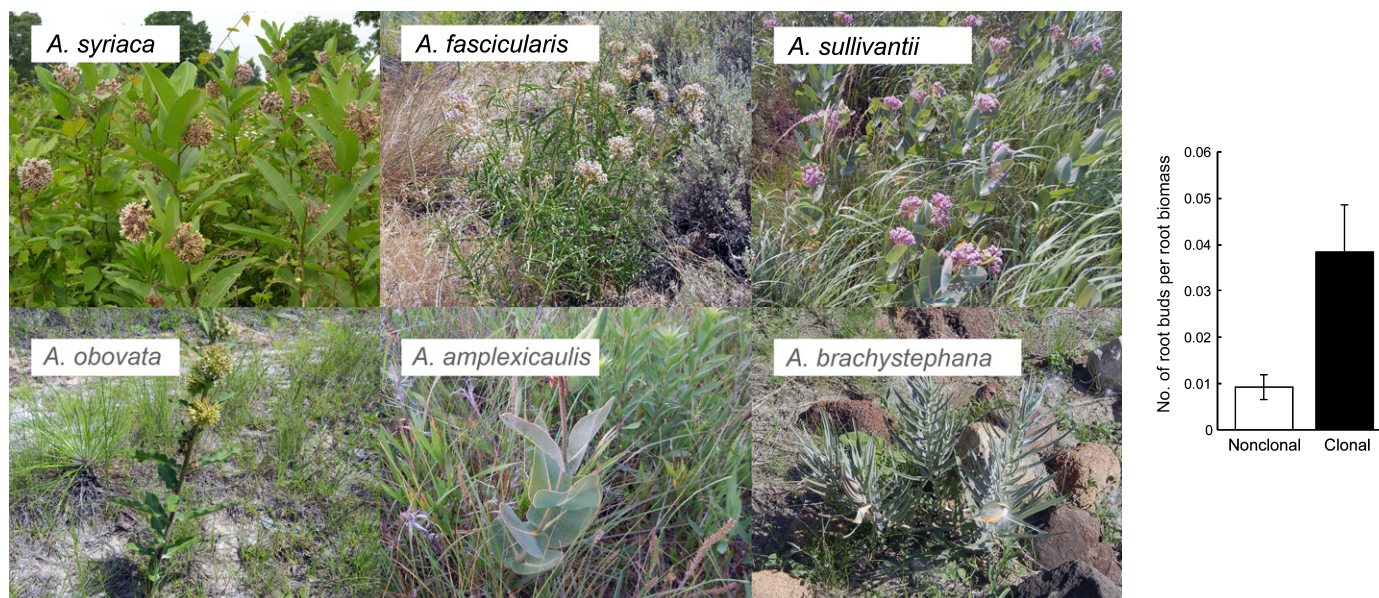
Fig. 2 Clonality and root buds in *Asclepias* species. Pictures of pairs of clonal species (upper row: *A. syriaca*, *A. fascicularis*, *A. sullivantii*), and nonclonal species (lower row: *A. obovata*, *A. amplexicaulis*, *A. brachystephana*). Pairs are based on similar numbers of root buds g^{-1} root mass (Supporting Information Table S1). The graph on the right side shows overall species averages ($\pm$ 1 SE) for the number of root buds per root biomass for clonal (closed bars), and nonclonal (open bars) species. Clonality was assigned by the authors according to growing habits in the field and during experimental manipulations. The difference in root buds between clonal and nonclonal species is highly significant (pGLS; $F_{1,48} = 12.39$, $P = 0.001$).

the distribution in the native range. We evaluated whether occurrences matched the described range of the species to exclude spurious records, as well as the record of species in the nonnative range. We extracted eight bioclimatic variables from Worldclim climate layers at a resolution of 2.5 arcminutes (Hijmans *et al.*, 2005): annual mean temperature (bio1), maximum temperature of the warmest month (bio5), minimum temperature of the coldest month (bio6), mean temperature of the warmest quarter (bio10), mean temperature of the coldest quarter (bio11), annual precipitation (bio12), precipitation of the warmest quarter (bio18), and precipitation of the coldest quarter (bio19). To explore the distribution of species and clonal strategies in the climatic space, we performed a principal component analysis (PCA) on species occurrences together with available environmental cells in the species range (including North and South America) as in Litsios *et al.* (2012). We used the dudi.pca function of the ade4 R package (Dray & Dufour, 2007). Because those variables were highly correlated to the first two PCA axes (see the Results section, and Table S2), we computed the mean and SD of the annual mean temperature and sum of precipitation for each of the species tested for further analyses.

We used a comprehensive phylogenetic tree of the monophyletic American clade of *Asclepias* (Fishbein *et al.*, 2011), and pruned the terminal taxa to our 49 study species (Fig. 1). We performed two phylogenetic ANOVAs (Blomberg *et al.*, 2003) as implemented in the R package 'Phytools' ver. 0.2.9 (Revell, 2012) to determine whether mean temperature and precipitation differed between clonal and nonclonal species. Then, we related the mean temperature and precipitation (explanatory variables) to constitutive defenses and inducibility (response variables) of defense using generalized least squares (GLS) as implemented in

the nlme package in R (Pinheiro, 2012). We fitted the models using restricted maximum likelihood (REML). We took into account phylogenetic nonindependence by using covariance structures derived from the phylogeny and assuming either an underlying Brownian motion (BM) process with off-diagonal elements being multiplied by the λ parameter to account for the extent of phylogenetic signal (Pagel, 1999), or an Orstein–Uhlenbeck (OU) process that models stabilizing selection with an attraction parameter α (Martins & Hansen, 1997). This was done with the ape R package (Paradis *et al.*, 2004) using the corPagel and corMartins functions. Both models were applied to account for potentially different evolutionary processes driving the evolution of the studied traits (Salamin *et al.*, 2010). We used Akaike information criterion (AIC) values to assess the fit of both covariance structures.

Rate of trait evolution

We compared the rate of evolution of defenses and climatic niche among clonal and nonclonal species. This measure describes the speed at which species explore the defense and climatic niche space. We used stochastic mapping (Huelsenbeck *et al.*, 2003) as implemented in the 'make.simmap' function of the R package 'Phytools' (Revell, 2012) to map probable realizations of the evolution of clonal trait on the consensus tree. We used the 'all rate different' model to evolve the clonality trait along the *Asclepias* phylogenetic tree and allowed different forward and backward rates between the two states, estimated the prior distribution of the states at the root of the tree and used the Markov chain Monte Carlo option to set the parameters of the Q transition matrix. To account for the inherent stochasticity of the process,

we performed 100 stochastic mapping replicates. We measured the differences in the rate of constitutive and inducibility of defense evolution, and the rate of climatic niche evolution, for clonal and nonclonal species by comparing the fit of a single rate BM model with that of a multiple rate model. Although it has been shown that other models fit the data better than the BM model, especially during adaptive radiations (Freckleton & Harvey, 2006; Agrawal *et al.*, 2009a), we chose to use BM because our goal was solely to compare the relative rate of evolution between groups and not the actual trait values. The single rate model assumes that all lineages accumulate the same amount of morphological variance per unit of time while the multiple model allows clonal species to have a different rate of evolution than the nonclonal species. Both models were specified in the Phytools package that implements the noncensored version of a typical analysis (O'Meara *et al.*, 2006). The best-fitting model was selected according to sample size corrected AIC (AICc) and a *P*-value obtained with a likelihood ratio test against the chi-squared distribution. We collected the *P*-values of the 100 replicates and summarized them by calculating their mean and median.

Results

Association between traits and niches

Species were distributed along two main environmental axes, temperature corresponding to the first PCA axis, and precipitations corresponding to the second PCA axes. We found that

Asclepias species occupied a subset of available environmental space and did not colonize the moistest and coldest climatic conditions. Nonclonal species showed a broader climatic niche than clonal ones, and especially occupied warmer and wetter climatic conditions. Annual mean temperature was strongly correlated to the first PCA axis (Pearson $R^2 = 0.96$), while annual total precipitation was strongly correlated to the second PCA axis (Pearson $R^2 = 0.81$). Therefore, we used those variables rather than more abstract PCA axis values, knowing that those two variables represent well the climatic space available to *Asclepias* sp.

Consistent with our predictions, we found that clonal species inhabit colder (phylogenetic ANOVA (pGLS), $P = 12.31$, $P = 0.003$) and drier ($P = 4.40$, $P = 0.045$) regions than nonclonal species (Fig. 3). However, contrary to predictions, clonal species occupy a more restricted climatic niche than nonclonal species (Fig. 3). Using pGLS, we found a positive relationship between temperature and constitutive cardenolides (coefficient $= 7.36$, $P = 0.04$, best model $=$ Pagel with $\lambda = 0.30$), and inducibility of cardenolides (coefficient $= 23.87$, $P < 0.01$, best model $=$ Pagel with $\lambda = 0.39$), indicating that species occupying colder conditions produce lower constitutive amounts and inducibility of these chemical defenses. Because it was previously shown that colder temperatures inhibit production of secondary metabolites (Pellissier *et al.*, 2014), and because tropical species were grown at slightly colder temperature than their natural growing conditions, we consider our results to be conservative. In other words, a traditionally high-cardenolide species like the tropical milkweed *Asclepias curassavica* (Malcolm, 1995), remains high when growing in the glasshouse (Rasmann & Agrawal,
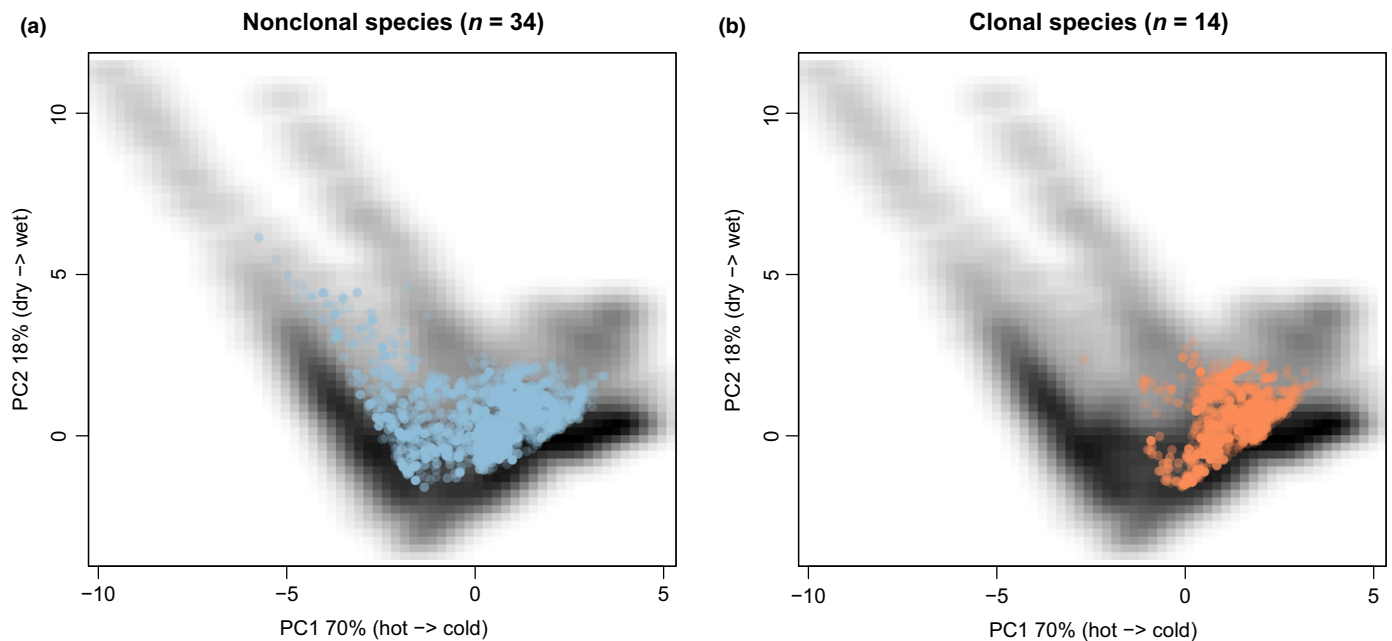

Fig. 3 Density plot from a principal component analysis of the climatic environment occupied by: (a) nonclonal *Asclepias* species (in blue; *A. angustifolia*, *A. barjoniifolia*, *A. californica*, *A. candida*, *A. humistrata*, *A. lanceolata*, *A. lemmonii*, *A. linaria*, *A. longifolia*, *A. Mexicana*, *A. perennis*, *A. pulchra*, *A. rubra*, *A. solanoana*, *A. subulata*, *A. texana*, *A. variegate*, *A. vestita*, *A. viridis*, *A. curassavica*, *A. incarnate*, *A. nivea*, *A. asperula*, *A. tuberosa*, *A. brachystephana*, *A. hirtella*, *A. cordifolia*, *A. boliviensis*, *A. quadrifolia*, *A. cryptoceras*, *A. engelmanniana*, *A. viridiflora*, *A. oenotheroides*, *A. amplexicaulis*, *A. obovata*, *A. glaucescens*); (b) clonal *Asclepias* species (in orange; *A. erosa*, *A. arenaria*, *A. sullivantii*, *A. eriocarpa*, *A. verticillata*, *A. exaltata*, *A. latifolia*, *A. lanuginose*, *A. purpurascens*, *A. hallii*, *A. speciosa*, *A. fascicularis*, *A. pumila*, *A. subverticillata*, *A. syriaca*, *A. ovalifolia*). The gray area indicates the potential environment available in both North and South America (Greenland excluded).

2011), while a low-cardenolide species like *Asclepias syriaca* produced similar concentrations in the glasshouse as in the field (Agrawal *et al.*, 2014).

Clonality showed negatively correlated evolution with cardenolides (Fig. 4). Using pGLS, we found that the number of subterranean buds per unit biomass correlated negatively with constitutive cardenolides (coefficient = −0.006, $P < 0.01$, best model = Pagel with $\lambda = 0$). We also found a similar negative relationship for inducibility of cardenolides, although Pagel and OU models were indistinguishable (Fig. 4, Pagel, coefficient = −0.01, $P = 0.04$, $\lambda = 0$; OU, coefficient = −0.01, $P = 0.07$, $\alpha = 12.20$; Table S3). Our qualitative categorization of clonality similarly indicated that clonal plant species have marginally lower constitutive concentrations of cardenolides after accounting for shared evolutionary history ($F = 4.64$, $P = 0.056$), while we detected no difference among clonal and nonclonal plants for inducibility ($F = 2.29$, P-value = 0.17, data not shown).

Rates of trait evolution

Modeling the rates of plant defense evolution showed an effect of clonality, in which nonclonal lineages evolve constitutive cardenolides almost 10 times faster than the clonal ones (Fig. 5; mean $\sigma 2$-clonal = 17.86, mean $\sigma 2$-nonclonal = 152.74, P-value of chi-squared test on AIC: mean = 0.048, median = 0.044), but not for inducibility of cardenolides (mean σ^2-clonal = 9.192, mean σ^2-nonclonal = 27.056, P-value of chi-squared test on AIC: mean = 0.24, median = 0.215).

Discussion

The investment in anti-herbivore defenses and variation in plant life-history traits in *Asclepias* are related to variation in climatic conditions into which the species have radiated. Clonal species (i.e. having plants with longer rhizomes and greater resprounting ability) are derived in *Asclepias* (Fig. 1), and this strategy is primarily observed in lower-temperature habitats. Clonal species

also showed reduced investment in chemical defense, suggesting that clonality, as a tolerance strategy, might trade off with chemical defense. Finally, clonal lineages showed a lower rate of chemical defense evolution compared with nonclonal ones. In sum, our results suggest that the evolution of clonality in environments with lower temperatures and precipitations goes hand in hand with a reduced rate of chemical defense evolution and reduced levels of chemical defense. This implies a direct effect of habitat selection on both reproduction type and resistance to herbivores.

Climatic niche, clonality, and chemical defenses

Deciphering the putative role of any functional trait in protecting plants against herbivores is complex, as traits may serve multiple functions across different environments. Clonal and nonclonal species showed not fully overlapping distributions along temperature and precipitation gradients, in which clonal species occupy a narrower, generally more stressful environment (i.e. drier and colder), suggesting that clonality might be better suited against abiotic stress (drought and cold) (Salzman, 1985; Santamaria, 2002; Weppler *et al.*, 2006). Similarly, Pellissier *et al.* (2010) found an increase in the use of clonality in high-elevation alpine plant communities, where both biotic (e.g. herbivores and pollinators) and abiotic conditions are more unstable compared with low-elevation sites (summarized in Rasmann *et al.*, 2014).

The evolution of high tolerance through clonal reproduction could also be an adaptation to biotic conditions if greater tolerance confers a competitive advantage in plant communities damaged by generalist grazing herbivores (e.g. grasslands McNaughton, 1979). Indeed, clonal species that form dense stands may be especially prone to concentrated herbivore attack (Root, 1973), thus favoring tolerance over chemical resistance traits. As a corollary, clonal species may be less frequent in warmer climates because this strategy is less successful against more specialized herbivore attack (Schemske *et al.*, 2009). Nevertheless, as was previously discussed by Agrawal *et al.* (2015), the reduction of cardenolides in more derived, cold-adapted species
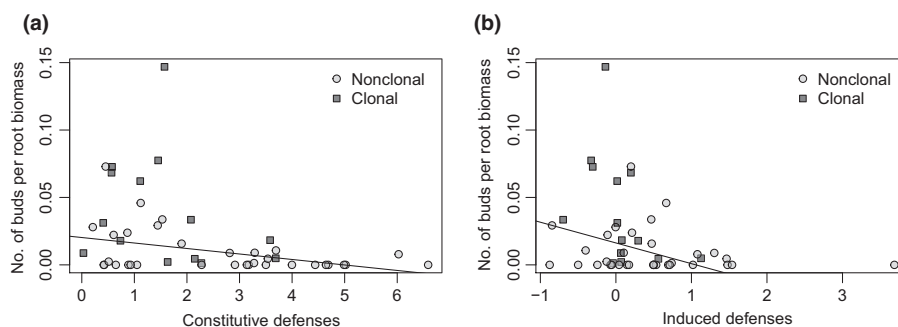

Fig. 4 Relationships between subterranean bud number per unit root biomass and constitutive (a) and induced (b) cardenolide defense. Each point represents species' means, and lines represent phylogenetically corrected linear regressions across all species. The clonality status of each species is indicated by a light circle for nonclonal *Asclepias* species (A. angustifolia, A. barjoniifolia, A. californica, A. candida, A. humistrata, A. lanceolata, A. lemmonii, A. linaria, A. longifolia, A. Mexicana, A. perennis, A. pulchra, A. rubra, A. solanoana, A. subulata, A. texana, A. variegate, A. vestita, A. viridis, A. curassavica, A. incarnate, A. nivea, A. asperula, A. tuberosa, A. brachystephana, A. hirtella, A. cordifolia, A. boliviensis, A. quadrifolia, A. cryptoceras, A. engelmanniana, A. viridiflora, A. oenotheroides, A. amplexicaulis, A. obovata, A. glaucescens), and a dark square for clonal *Asclepias* species (A. erosa, A. arenaria, A. sullivantii, A. eriocarpa, A. verticillata, A. exaltata, A. latifolia, A. lanuginose, A. purpurascens, A. hallii, A. speciosa, A. fascicularis, A. pumila, A. subverticillata, A. syriaca, A. ovalifolia).
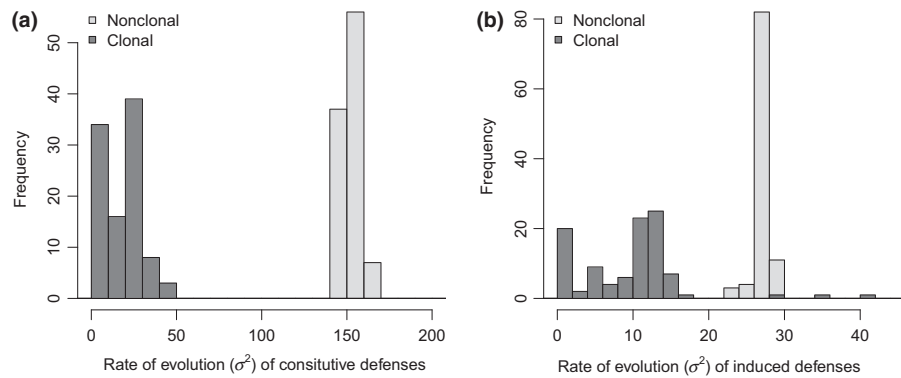
Fig. 5 Phylogenetic rates of constitutive (a) and induced (b) defense evolution. Rates are shown in dark gray for clonal *Asclepias* species (*A. erosa, A. arenaria, A. sullivantii, A. eriocarpa, A. verticillata, A. exaltata, A. latifolia, A. lanuginose, A. purpurascens, A. hallii, A. speciosa, A. fascicularis, A. pumila, A. subverticillata, A. syriaca, A. ovalifolia*), and light gray for nonclonal *Asclepias* species (*A. angustifolia, A. barjoniifolia, A. californica, A. candida, A. humistrata, A. lanceolata, A. lemmonii, A. linaria, A. longifolia, A. Mexicana, A. perennis, A. pulchra, A. rubra, A. solanoana, A. subulata, A. texana, A. variegate, A. vestita, A. viridis, A. curassavica, A. incarnate, A. nivea, A. asperula, A. tuberosa, A. brachystephana, A. hirtella, A. cordifolia, A. boliviensis, A. quadrifolia, A. cryptoceras, A. engelmanniana, A. viridiflora, A. oenotheroides, A. amplexicaulis, A. obovata, A. glaucescens*).

might be also influenced by other factors including sequestration by specialist herbivores, such as monarch butterflies, which are found throughout the range of *Asclepias* spp. Therefore, a combination of biotic and abiotic factors must have shaped the directional evolution of chemical defenses in *Asclepias* during their radiation toward the poles (Agrawal & Fishbein, 2008).

Tradeoffs between tolerance and resistance traits

In light of the increasing interest in investigating a broad range of functional traits that may be integrated into defense against herbivores (Carmona *et al.*, 2011), we found negative correlated evolution between stem bud-number and cardenolide production, suggestive of a tradeoff between resprouting abilities and defense.

The idea of resource-driven tradeoffs is central to the evolutionary theories of plant defenses (Zangerl & Bazzaz, 1992). The potential negative interactions between chemical defenses and tolerance have long been suggested (van der Meijden *et al.*, 1988; Núñez-Farfán *et al.*, 2007). Here, we found evidence of a negative correlation between resprouting ability and secondary metabolite production similar to previous studies. Indeed, while cardenolides and latex showed directional declines during the diversification of *Asclepias* (Agrawal *et al.*, 2008, 2009b), plant tolerance (i.e. the ability to regrow after defoliation) showed evidence of escalation (Agrawal & Fishbein, 2008). Additionally, it was also observed that species with higher numbers of root buds, have higher regrowth capacity after damage (i.e. tolerance) (Agrawal *et al.*, 2015), indicating that more clonal species have higher tolerance capacity, while decreasing chemical defenses.

The negative correlation between defense and tolerance could reflect allocation costs of the traits as well as trait redundancy similar to the growth defense tradeoff (Herms & Mattson, 1992; Fine *et al.*, 2006; Lind *et al.*, 2013). Puijalon *et al.* (2011) recently showed a negative correlation between a plant's ability to avoid mechanical stress or to be able to tolerate it, again suggestive of a tradeoff between alternative strategies. As suggested earlier, in *Asclepias*, the strategy of producing new clonal ramets might be

sufficient to compensate low cardenolide production against above-ground damage (Agrawal *et al.*, 2015), and this may be particularly true for milkweeds as they are mainly confronted with specialist, cardenolide-adapted, herbivores (Agrawal *et al.*, 2012). That said, other unmeasured factors correlating with both clonality and chemical defenses might drive the observed pattern, and future work is needed to tease apart the effect of biotic factors (herbivore pressure and herbivore sequestration) and abiotic factors (climate, soil conditions) in driving resprouting abilities and chemical defenses.

Rate of defense evolution and clonality along the climatic niche

We observed a higher rate of chemical defense evolution in nonclonal than in clonal species. Variation in the rate of evolution for a particular trait is influenced by several factors, including the rate of sexual reproduction (Johnson *et al.*, 2009) and the selective pressure on a particular trait. It has been argued that the longer generation time and less frequent recombination of clonal species lead to slowed genetic change and decreased rate of trait evolution (Hamilton *et al.*, 1990; Charlesworth & Wright, 2001; Godfrey & Johnson, 2014). Additionally, generation time is negatively correlated with molecular substitution rates in angiosperms, allowing typically short-lived herbaceous species to 'explore' a wider climatic space than longer-lived species because of higher rates of evolution for climatic preference (Smith & Beaulieu, 2009). In accordance with these ideas, we observed that the derived clonal trait in *Asclepias* spp. (Agrawal *et al.*, 2015) only evolved in colder conditions, and in concordance with previous finding; the derived species invest less in chemical defenses (Agrawal *et al.*, 2008, 2009b); and the rate of change of defensive traits was most rapid early in the radiation of *Asclepias* spp. (Agrawal *et al.*, 2009a). Taken together, our findings support the hypothesis that radiation of *Asclepias* spp. into colder climates was accompanied by a proliferation of more clonal species that are, in turn, less chemically defended. As would be postulated for

lineages with greater usage of asexual modes of reproduction (Johnson *et al.*, 2009), we found a lower rate of defense evolution in more clonal lineages. Interestingly, though, as all *Asclepias* are perennial, clonality *per se* seems to drive the observed pattern of defense evolution, and not a dramatic change from annual to perennial mode of existence.

Conclusions

The recent advances in phylogenetic and comparative analyses allow the emergence of a clearer scenario regarding which factors drive the ecology and evolution of plant defenses. Our study, along with recent work (Fine *et al.*, 2006; Rasmann & Agrawal, 2011; Pearse & Hipp, 2012; Pellissier *et al.*, 2013; Moreira *et al.*, 2014), highlights a strong effect of both biotic and abiotic factors on the evolution of plant resistance. Overall, plants growing in warmer and more humid climates, where herbivore pressure is high, invest more in chemical defenses than those in colder climates. Moreover, clonality and resistance abilities trade off among species, but this relationship is affected by differences in the climatic niche of the species (i.e. more tropical species are less clonal, and invest more in defenses, whereas the opposite is true for more temperate species). Future work will be needed to fully tease apart selection by herbivores at different latitudes and climate variables on defense production, as colder temperatures *per se* may be sufficient for inhibiting defense (Pellissier *et al.*, 2014). The modes of induction and interactions with other resistance strategies may be dictated by resource-driven, physiological and evolutionary tradeoffs, which, in turn, are also influenced by biotic and abiotic factors of the niche (Coley *et al.*, 1985; Mooney *et al.*, 2010).

Author contributions

A.A.A., and S.R. planned and designed the research. A.A.A. and S.R. performed experiments, and chemical analyses. L.P., G.L., S.R. and M.F. analyzed the data. S.R., L.P., G.L., A.A.A., M.F. and N.S. wrote the manuscript.

References

Agrawal AA, Ali JG, Rasmann S, Fishbein M. 2015. Macroevolutionary trends in the defense of milkweeds against monarchs: latex, cardenolides, and tolerance of herbivory. In: Oberhauser K, Altizer S, Nail K, eds. *Monarchs in a changing world: biology and conservation of an iconic insect.* Ithaca, NY, USA: Cornell University Press, 47–59.

Agrawal AA, Fishbein M. 2008. Phylogenetic escalation and decline of plant defense strategies. *Proceedings of the National Academy of Sciences, USA* **105**: 10057–10060.

Agrawal AA, Fishbein M, Halitschke R, Hastings AP, Rabosky DL, Rasmann S. 2009a. Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proceedings of the National Academy of Sciences, USA* **106**: 18067–18072.

Agrawal AA, Lajeunesse MJ, Fishbein M. 2008. Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata* **128**: 126–138.

Agrawal AA, Patrick ET, Hastings AP. 2014. Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere* **5**: art126.

Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S. 2012. Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytologist* **194**: 28–45.

Agrawal AA, Salminen JP, Fishbein M. 2009b. Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution* **63**: 663–673.

Belsky AJ, Carson WP, Jensen CL, Fox GA. 1993. Overcompensation by plants – herbivores optimization or red herring. *Evolutionary Ecology* **7**: 109–121.

Blomberg SP, Garland T, Ives AR, Crespi B. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Bond WJ, Midgley JJ. 2001. Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology & Evolution* **16**: 45–51.

Brower LP, Kl Williams, McEvoy PB, Flannery MA. 1972. Variation in cardiac glycoside content of monarch butterflies from natural populations in Eastern North-America. *Science* **177**: 426–429.

Carmona D, Lajeunesse MJ, Johnson MTJ. 2011. Plant traits that predict resistance to herbivores. *Functional Ecology* **25**: 358–367.

Charlesworth D, Wright SI. 2001. Breeding systems and genome evolution. *Current Opinion in Genetics & Development* **11**: 685–690.

Coley PD, Aide TM. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. In: Price PW, Lewinsohn TM, Fernandes GW, Benson WW, eds. *Plant–animal interactions: evolutionary ecology in tropical and temperate regions.* New York, NY, USA: Wiley, 25–49.

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *Science* **230**: 895–899.

Currano ED, Labandeira CC, Wilf P. 2010. Fossil insect folivory tracks paleotemperature for six million years. *Ecological Monographs* **80**: 547–567.

Dobler S, Dalla S, Wagschal V, Agrawal AA. 2012. Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na, K-ATPase. *Proceedings of the National Academy of Sciences, USA* **109**: 13040–13045.

Dray S, Dufour AB. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**: 1–20.

Endara MJ, Coley PD. 2011. The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology* **25**: 389–398.

Fahrig L, Coffin DP, Lauenroth WK, Shugart HH. 1994. The advantage of long-distance clonal spreading in highly disturbed habitats. *Evolutionary Ecology* **8**: 172–187.

Fine PVA, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Saaksjarvi I, Schultz LC, Coley PD. 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* **87**: S150–S162.

Fischer M, Stocklin J. 1997. Local extinctions of plants in remnants of extensively used calcareous grasslands 1950–1985. *Conservation Biology* **11**: 727–737.

Fishbein M, Chuba D, Ellison C, Mason-Gamer RJ, Lynch SP. 2011.
Phylogenetic relationships of *Asclepias* (Apocynaceae) inferred from non-coding
chloroplast DNA sequences. *Systematic Botany* **36**: 1008–1023.

Freckleton RP, Harvey PH. 2006. Detecting non-Brownian trait evolution in
adaptive radiations. *PLoS Biology* **4**: 2104–2111.

Godfrey RM, Johnson MTJ. 2014. Effects of functionally asexual reproduction
on quantitative genetic variation in the eventing primroses (*Oenothera*,
Onagraceae). *American Journal of Botany* **101**: 1906–1914.

Hamilton WD, Axelrod R, Tanese R. 1990. Sexual reproduction as an
adaptation to resist parasites (a review). *Proceedings of the National Academy of
Sciences, USA* **87**: 3566–3573.

Herms DA, Mattson WJ. 1992. The dilemma of plants – to grow or defend.
Quarterly Review of Biology **67**: 283–335.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high
resolution interpolated climate surfaces for global land areas. *International
Journal of Climatology* **25**: 1965–1978.

Huelsenbeck JP, Nielsen R, Bollback JP. 2003. Stochastic mapping of
morphological characters. *Systematic Biology* **52**: 131–158.

Johnson MTJ, Smith SD, Rausher MD. 2009. Plant sex and the evolution of
plant defenses against herbivores. *Proceedings of the National Academy of
Sciences, USA* **106**: 18079–18084.

de Jong TJ, van der Meijden E. 2000. On the correlation between allocation to
defence and regrowth in plants. *Oikos* **88**: 503–508.

Lankau RA. 2007. Specialist and generalist herbivores exert opposing selection on
a chemical defense. *New Phytologist* **175**: 176–184.

Levin DA, York BM. 1978. Toxicity of plant alkaloids – ecogeographic
perspective. *Biochemical Systematics and Ecology* **6**: 61–76.

Lewinsohn TM. 1991. The geographical distribution of plant latex. *Chemoecology*
2: 64–68.

Lind EM, Borer E, Seabloom E, Adler P, Bakker JD, Blumenthal DM, Crawley
M, Davies K, Firn J, Gruner DS *et al.* 2013. Life-history constraints in
grassland plant species: a growth-defence trade-off is the norm. *Ecology Letters*
16: 513–521.

Litsios G, Pellissier L, Forest F, Lexer C, Pearman PB, Zimmermann NE,
Salamin N. 2012. Trophic specialization influences the rate of environmental
niche evolution in damselfishes (Pomacentridae). *Proceedings of the Royal Society
of London B: Biological Sciences* **279**: 3662–3669.

Litsios G, Wuest RO, Kostikova A, Forest F, Lexer C, Linder HP, Pearman PB,
Zimmermann NE, Salamin N. 2014. Effects of a fire response trait on
diversification in replicated radiations. *Evolution* **68**: 453–465.

Malcolm SB. 1991. Cardenolide-mediated interactions between plants and
herbivores. In: Rosenthal GA, Berenbaum MR, eds. *Herbivores: their
interactions with secondary metabolites*. San Diego, CA, USA: Academic Press,
251–296.

Malcolm SB. 1995. Milkweeds, monarch butterflies and the ecological
significance of cardenolides. *Chemoecology* **5/6**: 101–117.

Marquis RJ. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science*
226: 537–539.

Martins EP, Hansen TF. 1997. Phylogenies and the comparative method: a
general approach to incorporating phylogenetic information into the analysis of
interspecific data. *American Naturalist* **149**: 646–667.

McNaughton SJ. 1979. Grazing as an optimization process – grass ungulate
relationships in the Serengeti. *American Naturalist* **113**: 691–703.

van der Meijden E, Wijn M, Verkaar HJ. 1988. Defence and regrowth, alternative
plant strategies in the struggle against herbivores. *Oikos* **51**: 355–363.

Moles AT, Bonser SP, Poore AGB, Wallis IR, Foley WJ. 2011. Assessing the
evidence for latitudinal gradients in plant defence and herbivory. *Functional
Ecology* **25**: 380–388.

Mooney KA, Halitschke R, Kessler A, Agrawal AA. 2010. Evolutionary trade-
offs in plants mediate the strength of trophic cascades. *Science* **327**: 1642–1644.

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R,
Sampedro L. 2014. Trade-offs between constitutive and induced defences drive
geographical and climatic clines in pine chemical defences. *Ecology Letters* **17**:
537–546.

Núñez-Farfán J, Fornoni J, Valverde PL. 2007. The evolution of resistance and
tolerance to herbivores. *Annual Review of Ecology Evolution and Systematics* **38**:
541–566.

O'Meara BC, Ane C, Sanderson MJ, Wainwright PC. 2006. Testing for
different rates of continuous trait evolution using likelihood. *Evolution* **60**:
922–933.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N. 2013. *caper:
comparative analyses of phylogenetics and evolution in R*. URL https://cran.r-
project.org/web/packages/caper/index.html [accessed 19 February 2015].

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature*
401: 877–884.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and
evolution in R language. *Bioinformatics* **20**: 289–290.

Pearse IS, Hipp AL. 2012. Global patterns of leaf defenses in oak species.
Evolution **66**: 2272–2286.

Pellissier L, Fiedler K, Ndribe C, Dubuis A, Pradervand J-N, Guisan A,
Rasmann S. 2012. Shifts in species richness, herbivore specialization, and plant
resistance along elevation gradients. *Ecology and Evolution* **2**: 1818–1825.

Pellissier L, Fournier B, Guisan A, Vittoz P. 2010. Plant traits co-vary with altitude
in grasslands and forests in the European Alps. *Plant Ecology* **211**: 351–365.

Pellissier L, Ndiribe C, Dubuis A, Pradervand J-N, Salamin N, Guisan A,
Rasmann S. 2013. Turnover of plant lineages shapes herbivore phylogenetic
beta diversity along ecological gradients. *Ecology Letters* **16**: 600–608.

Pellissier L, Roger A, Bilat J, Rasmann S. 2014. High elevation *Plantago
lanceolata* plants are less resistant to herbivory than their low elevation
conspecifics: is it just temperature? *Ecography* **37**: 950–959.

Pennings SC, Callaway RM. 2000. The advantages of clonal integration under
different ecological conditions: a community-wide test. *Ecology* **81**: 709–716.

Pennings SC, Ho C-K, Salgado CS, Wieski K, Davé N, Kunza AE, Wason EL.
2009. Latitudinal variation in herbivore pressure in Atlantic Coast salt marshes.
Ecology **90**: 183–195.

Pinheiro J. 2012. *Package 'nlme': linear and nonlinear mixed effects models*. URL
https://cran.r-project.org/web/packages/nlme/index.html [accessed 19 August
2015].

Puijalon S, Bouma TJ, Douady CJ, van Groenendael J, Anten NPR, Martel E,
Bornette G. 2011. Plant resistance to mechanical stress: evidence of an
avoidance-tolerance trade-off. *New Phytologist* **191**: 1141–1149.

Rasmann S, Agrawal AA. 2011. Latitudinal patterns in plant defense: evolution
of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*
14: 476–483.

Rasmann S, Alvarez N, Pellissier L 2014. The altitudinal niche-breadth
hypothesis in insect–plant interactions. In: Voelckel C, Jander G eds. *Annual
plant reviews, vol. 47, insect–plant interactions*. Chichester, UK: John Wiley &
Sons Ltd, 339–359.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology
(and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Root RB. 1973. Organization of a plant–arthropod association in simple and
diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*
43: 95–124.

Salamin N, Wueest RO, Lavergne S, Thuiller W, Pearman PB. 2010. Assessing
rapid evolution in a changing environment. *Trends in Ecology & Evolution* **25**:
692–698.

Salzman AG. 1985. Habitat selection in a clonal plant. *Science* **228**: 603–604.

Santamaria L. 2002. Why are most aquatic plants widely distributed? Dispersal,
clonal growth and small-scale heterogeneity in a stressful environment. *Acta
Oecologica-International Journal of Ecology* **23**: 137–154.

Schemske DW. 2009. Biotic interactions and speciation in the tropics. In: Butlin
RK, Bridle JR, Schluter D, eds. *Speciation and patterns of diversity*. Cambridge,
UK: Cambridge University Press, 219–239.

Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K. 2009. Is there a
latitudinal gradient in the importance of biotic interactions? *Annual Review of
Ecology Evolution and Systematics* **40**: 245–269.

Schmid B, Puttick GM, Burgess KH, Bazzaz FA. 1988a. Clonal integration
and effects of simulated herbivory in old-field perennials. *Oecologia* **75**:
465–471.

Schmid B, Puttick GM, Burgess KH, Bazzaz FA. 1988b. Clonal integration
and effects on simulated herbivory in old-field perennials. *Oecologia* **75**:
465–471.

Schoonhoven LM, van Loon JJA, Dicke M. 2005. *Insect–plant biology*. Oxford,
UK: Oxford University Press.

Smith SA, Beaulieu JM. 2009. Life history influences rates of climatic niche evolution in flowering plants. *Proceedings of the Royal Society B: Biological Sciences* **276**: 4345–4352.

Stowe KA, Marquis RJ, Hochwender CG, Simms EL. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics* **31**: 565–595.

Strauss SY, Rudgers JA, Lau JA, Irwin RE. 2002. Direct and ecological costs of resistance to herbivory. *Trends in Ecology & Evolution* **17**: 278–285.

Strong DR, Lawton JH, Southwood R. 1984. *Insects on plants: community patterns and mechanisms*. London, UK: Blackwell Scientific.

Weppler T, Stoll P, Stocklin J. 2006. The relative importance of sexual and clonal reproduction for population growth in the long-lived alpine plant *Geum reptans*. *Journal of Ecology* **94**: 869–879.

Wise MJ, Abrahamson WG. 2005. Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. *Oikos* **109**: 417–428.

de Witte LC, Armbruster GFJ, Gielly L, Taberlet P, Stoecklin J. 2012. AFLP markers reveal high clonal diversity and extreme longevity in four key arctic-alpine species. *Molecular Ecology* **21**: 1081–1097.

de Witte LC, Stoecklin J. 2010. Longevity of clonal plants: why it matters and how to measure it. *Annals of Botany* **106**: 859–870.

Woodson RE. 1954. The North American species of *Asclepias* L. *Annals of the Missouri Botanical Garden* **41**: 1–211.

Zalucki MP, Brower LP, Alonso A. 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology* **26**: 212–224.

Zangerl AR, Bazzaz FA. 1992. Theory and pattern in plant defense allocation. In: Fritz RS, Simms EL, eds. *Plant resistance to herbivores and pathogens: ecology, evolution and genetics*. Chicago, IL, USA: University of Chicago Press, 363–391.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Clonality table of 49 *Asclepias* species

Table S2 Loading values for the PCA analysis

Table S3 Results of the pGLS analyses

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <27 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**