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Original research

Developmental constraints and resource environment shape early emergence and investment in spines in saplings

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Running title: Early emergence and investment in spines in saplings

- **Background and Aims** Herbivory by large mammals imposes a critical recruitment bottleneck on plants in many systems. Spines defend plants against large herbivores and how early they emerge in saplings may be one of the strongest predictors of sapling survival in herbivore-rich environments. Yet little effort has been directed at understanding the variability in spine emergence across saplings.

- **Methods** We present a multi-species study examining whether and how sapling size, spine type and species' environmental niche (light and precipitation environment) influence early emergence and biomass investment in spines. A phylogenetically diverse pool of 45 species possessing different spine types (spines, prickles and thorns; that are derived from distinct plant organs: leaf, epidermis or cortex, and branch, respectively), were grown under common garden conditions, and patterns of spine emergence and biomass allocation to spines at 5 and 15 weeks after transplanting were characterized.
- **Key Results** Spine type and species' resource niche were the main factors driving early emergence and investment patterns. Spines emerged earliest in leaf spine-bearing species, and latest in thorn-bearing species. The probability of early spine emergence increased with decreasing precipitation, and was greater in species from open than closed habitats. Sapling investment in spines changed with plant mass but was contingent on spine type and habitat type.
- **Conclusions** Different spine types have strikingly different timing of expression, suggesting that developmental origins of spines play a critical role in sapling defences. Furthermore, species from different precipitation and light environments (open vs. closed habitats) showed contrasting patterns of early spine expression suggesting that resource limitation in their native range may have driven divergent evolution of early defence expression.

Key words: developmental constraints, early emergence, large herbivore, prickle, resource environment, sapling, spine, thorn, spinescence.

INTRODUCTION

In systems where large mammalian herbivores (hereafter, large herbivores) are abundant, they have severe impacts, via persistent defoliation, on the survival and growth of woody plants at juvenile stages (Bond 2008; Staver and Bond 2014; Churski *et al.* 2017). Herbivory by smaller browsing and mixed-feeding mammals on seedlings and saplings represents a significant bottleneck in tree recruitment in herbivore-rich systems (Prins and van der Jeugd 1993; Staver and Bond 2014). In these systems, being defended early in development is potentially crucial for tree survival. A growing body of literature has revealed spines to be an essential and specific structural defence against small to large herbivores, particularly in adult plants (Cooper and Owen-smith 1986; Shipley 2007; Hanley *et al.* 2007; Charles-Dominique *et al.* 2017). Spines (like most structural defences) become more important in plant defence during the sapling stages (Hanley *et al.* 2007; Barton and Koricheva 2010; Ochoa-López *et al.* 2015). Even though deployment of spines in saplings varies substantially across species (Grubb 1992), little effort has been directed at understanding the sources of this variation. Understanding variability among species in the early expression of spines could provide insights into survivorship of spiny saplings and help to explain their distribution across environmental gradients (Myers and Bazely 1991; Grubb 1992; Moles *et al.* 2011; Schmidt *et al.* 2013; Tindall *et al.* 2016).

Here, we examined early emergence and investment in spines in saplings across a large number of species. We describe the constitutive onset of spines across species from different habitats. Plant defence theories posit that variation in defence expression across species is driven by differences in plant growth rate (Herms and Mattson 1992), environmental resource supply (Coley *et al.* 1985), plant internal resource pools (Bryant *et al.* 1983) and risk of herbivory (McKey 1974; Rhoades 1979). However, differences in expression of physical defences (e.g.

pubescence, spines) may also be partly explained by differences in the origin of the plant tissue/organ modified (Hanley *et al.* 2007; Barton 2016), but this source of variation is currently not accounted for by plant defence theories (Barton and Boege 2017). We formulated an integrative framework (**Fig. 1**) that incorporates predictions from both plant defence (Stamp 2003) and developmental constraints theories (Barton and Boege 2017) to examine early spine expression in saplings.

Spines can be produced from different plant organs (Bell and Bryan 2008) that could impose a strong developmental constraint on their expression in saplings (**Fig.1a**). Spines, derived from modified leaves and leaf parts are likely to incur significant cost in terms of lost photosynthesis, but may be the earliest to emerge (Fig.1b, shown with “1”) as they can be deployed simultaneously to the growth of first leaves. Prickles, derived from outgrowths of the epidermis or cortex, have lower construction costs (Bazely *et al.* 1991), but their emergence may be delayed because they undergo longer developmental phases (Kellogg *et al.* 2011; Gallenmüller *et al.* 2015). Thorns, derived from stems and auxiliary meristems, should be slowest to emerge as early plant growth involves first the development of the primary stem (and associated leaves) before lateral branching can occur to form thorns (Barthelemy and Caraglio 2007; Bell and Bryan 2008). Further, building thorns involves activation and lignification of auxiliary meristems which require substantial biomass investment and are therefore likely to be affected by overall plant size to a greater extent than spines or prickles. In addition to these allocation cost and anatomical constraints, the timing of spine expression may reflect adaptation to resource availability.

Plant defence theory generally predicts trade-off between growth and defence investment (Stamp 2003) either due to resource allocation constraints (Herms and Mattson 1992) or shared

plant regulatory pathways (Campos *et al.* 2016; Zuest and Agrawal 2017). Spines are thought to incur allocation costs (Craine *et al.* 2003) by diverting carbon pools from building other plant vegetative parts (e.g. stem, leaves) (Skogsmyr and Fagerstom 1992). Importantly, unlike many chemical defences, allocation to spines represents fixed investment that cannot be recycled (Grubb 1992). For young saplings, such fixed investment may be particularly costly due to limited total resource pools (Boege and Marquis 2005). Given that realized plant size (i.e. total biomass) reflects growth rate (e.g. fast-growing species produce bigger saplings) and absolute internal resource pools (Bazzaz *et al.* 1987), species producing bigger saplings (i.e. those with fast biomass accumulation rate) are likely selected for lower constitutive investment in spines (Fig. 1c). In contrast, slow-growing species (producing smaller saplings) may invest highly in spines to reduce loss of limited resources (Coley *et al.* 1985; Herms and Mattson 1992).

Species from resource-limited environments generally grow slower and have low post-defoliation regrowth capacity and are therefore expected to invest heavily in constitutive defences (Coley *et al.* 1985; Swihart and Bryant 2001; Stamp 2003). Thus, we predicted that saplings of spiny species from low precipitation and light environments are likely selected for greater expression of spines relative to those from high precipitation and light environments (Fig. 1d-f). Indeed the general distribution of spiny plants suggests that environments characterized by low precipitation may select for greater incidence of spines (Grubb 1992; Charles-Dominique *et al.* 2016). For instance, the proportion of spiny plants generally increases with decreasing rainfall (Grubb 1992; Schmidt *et al.* 2013). However, the distribution of spinescence across light gradients is in contrast to the expectation of greater spinescence under low light conditions. Spiny plants tend to be more common in ‘open’ than ‘closed’ (e.g. forests) habitats (Myers and Bazely 1991; Grubb 1992; Charles-Dominique *et al.* 2016; Osborne *et al.* 2018). However, it

remains unknown if saplings of spiny species from closed habitat (i.e. low light niche) have been selected for early and greater investment in spines than those from open habitats (i.e. high light niche).

To understand whether spiny plants with different spine types or from distinct environments have evolved different strategies for early spine expression (in order to survive the limitations encountered in their native environments), we sampled 45 spinescent species across the angiosperm phylogeny that naturally occur under diverse environments and grew them under common garden conditions. We then assessed constitutive expression of spines (i.e. baseline defence expression) by quantifying emergence timing and biomass investment in spines at two temporal periods (at 5 and 15 weeks after transplanting). We specifically tested the following four predictions: 1) Sapling size is negatively related to emergence time and biomass investment in spines, reflecting growth-defense trade-offs due to allocation costs; 2) Spines (i.e. leaf and stipular spines) and prickles are the fastest to emerge (because leaves are produced immediately upon germination and prickles generally incur low biomass investment) while thorns are likely to be delayed due to architectural constraints in saplings and their expression is potentially tied to sapling size. Across species, emergence and investment in spines: 3) scales negatively with mean annual precipitation and 4) is greater and independent of sapling size for species from closed than open environment potentially reflecting the cost of herbivory associated with plants growing under more arid and lower light conditions.

MATERIAL AND METHODS

Seed collection and germination

We sampled seeds of spiny species from the living collections of Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Science (XTBG-CAS), located in Menglun, Yunnan, China (21° 55' 38" N, 101°15'6" E); the XTBG-CAS savanna field station in Yuanjiang Valley, Yunnan (23° 28' 15" N, 103°10' 37" E); the Germplasm Bank of Wild Species, Kunming Institute of Botany (KIB-CAS) and from South Africa by Tomlinson et al. (Tomlinson et al. 2012). In total we germinated seeds of forty-five species that were mostly natives to tropical China (10 species), Southeast Asia (15 species) and Africa (13 species; see Supplementary data Table S1 for details) and distributed across wet to arid and closed to open habitats.

We classified species according to their spine types as: stipular spines, leaf spines, thorns, or prickles (Grubb 1992; Gutschick 1999; Bell and Bryan 2008). Here, we placed *Berberis* species in the ‘leaf spines’ group to differentiate them from ‘stipular spines’ because spines in *Berberis* are produced from both leaves and stipules. Our sample included species belonging to 17 plant families (Supplementary data Table S1), of which 13 species (6 families) possess prickles, 21 species (12 families) possess thorns, five species (all *Berberis*) possess leaf spines, and six species (2 families) possess stipular spines. We initially attempted to germinate additional leaf-spiny species of *Ilex* (e.g. *I. cornuta* and *I. aquifolium*) and *Hakea* (*H. oleifolia*, *H. erinacea* and *H. prostrata*) but were unsuccessful.

We grouped species into ‘closed’ (forest; 16 species) or ‘open’ habitats (savanna, thicket, grassland; 29 species) based on descriptions from the online flora covering the geographic distribution of the species (e.g. Flora of China: <http://efloras.org/>; <http://worldwidewattle.com/>) as well as published references (Coates-Palgrave 2002). We complemented this with expert opinion for species with widespread distributions. For thorns and prickles, we have representative species in both open and closed habitats, but all stipular and leaf spine species are

from open habitats only (Supplementary data Table S1).

We derived mean annual precipitation (MAP) per species based on the distribution range of each species. We determined the MAP for each species using species distribution data (GBIF.org: <http://www.gbif.org/occurrence>; 8 August 2017) and combined it with precipitation data produced by Fick et al., (2017; WorldClim:<http://worldclim.org/version2>) using the zonal statistics tool in ArcMap desktop GIS (version 10.2, Esri Inc. CA, USA).

Greenhouse Experiment

Seeds were germinated either on agar or river sand and after 10 days transplanted into a greenhouse located in XTBG-CAS for the experiment. The greenhouse was covered with shade netting from March to October to reduce irradiance levels to 40 – 50 % of full sunlight. This was necessary to reduce desiccation and mortality of the young seedlings, particularly of the forest species. Temperature and relative humidity in the greenhouse ranged over the course of the study from 19.7 – 33.3°C and 42 – 100% respectively. At the time of transplanting, each seedling was placed in a plastic tube of 10 cm diameter and 80 cm length. We chose deep pots to allow adequate space for taproot growth and reduce pot-binding effects, particularly for species from open habitats (Tomlinson *et al.* 2012). Tubes were filled with river sand mixed with Osmocote 18-6-12 N-P-K fertilizer (8-9 month mixture) at a concentration of 5kg of fertilizer per m⁻³ of river sand. Each tube was irrigated for one minute two times daily using an automated irrigation system (EZ Pro™ Jr, Signature Control Systems Inc., Peoria, USA).

Transplanted seedlings were allowed to grow for a minimum of 5 (ranged between 5-8 weeks) and 15 (ranged between 15 – 19 weeks) weeks before harvesting (hereafter week 5 and 15 respectively) for trait measurements. Here, we referred to the post 5 weeks plants as

‘saplings’ because the 4th-5th leaves had emerged on most plants and cotyledons had dropped in most species suggesting that these plants were no longer dependent on stored energy reserves from the cotyledons (Hanley *et al.* 2004; Barton and Hanley 2013), and thus capturing a potential shift in resource status for many species. Selection for early or late defence expression should be most apparent at week 5 given that plants have just transitioned from the seedling to sapling stage and have limited resource pools (Boege and Marquis 2005). At week 15, sapling should generally have more resources to allow for investment in defence. Where possible, we harvested at least 5 individuals per species at each harvest date. However, for 13 species (including all species with leaf spines), we did not have enough individuals and therefore harvested all individuals of these species at week 15. The greenhouse experiment was conducted in two temporal blocks from July – December in both 2015 and 2016. Thirteen species (3 prickles, 5 stipular spines and 5 thorns) were grown in 2015, whereas 32 species (5 leaf spines, 10 prickles, 1 stipular spines and 16 thorns) were grown in 2016 (Supplementary data Table S1). Greenhouse conditions (minimum, mean and maximum temperature and relative humidity) did not differ between 2015 and 2016 ($p > 0.05$) and preliminary analyses showed that there was no need to include “Year” as a random factor in the models described below.

Trait measurements

We characterized early spine expression using both the presence of spines on saplings and the biomass invested in spines. We inspected and recorded the presence of spines as our measure of spine emergence (yes=1 and no=0) on each individual at week 5. At week 15, we again checked the presence of spines on each individual (for those that did not produce spines at week 5).

We carefully removed all spines borne on all plant organs to estimate spine mass fraction (SPMF, our second response variable). Spine mass fraction is commonly reported as a measure of biomass investment in defence (Bazely *et al.* 1991; Gowda 1997; Pisani and Distel 1998; Craine *et al.* 2003; Gowda and Palo 2003). Here, we preferred SPMF over other measures of investment in spines (e.g. spine length or density) because it gives an indication of biomass allocation cost that can easily be related to total investment in other plant parts such as leaves (Skogsmyr and Fagerstrom 1992; Craine *et al.* 2003). SPMF was estimated only at week 15 as spines were too small to allow for accurate estimation of spine mass at week 5. In all cases, we detached spines from the plant organ using either sharp razor blades or utility knives. For estimation of thorn mass, we included only modified branches with pointed and lignified tips (most species that produced thorns had only thorns emerging and few true branches that were clearly different). We thus removed thorns from the base (attached to the primary stem). The leaf spine species in our dataset often produced whole-leaf modified spines or produced clearly visible spines from modified leaf-tips. For species with prickles, we removed prickles from both the leaves and stem.

Biomass of all plants harvested at any of the two sampling dates was divided into leaf, stem, and root tissues, dried to constant weight and weighed. We added the leaf, stem, root, and spine tissue masses to obtain the total sapling biomass (Mass, g, our variable for plant size) and subsequently determined organ mass fractions. We computed the spine mass fraction (SPMF, g g^{-1} , measured only during week 15 harvesting) as the ratio of spine mass to sapling mass for each individual.

Data analyses

Given the number of species in our dataset, and the possibility that phylogeny may influence the examined relationships, we conducted both ordinary and phylogenetically-adjusted analyses (Garamszegi 2014; Lajeunesse and Fox 2015). Phylogenetically-adjusted models were used here to assess whether our conclusions were influenced by the sampled taxa and not to infer any evolutionary pattern. Both phylogenetic and non-phylogenetic models produced qualitatively similar results. We therefore, for simplicity, describe the analyses without phylogenetic corrections here. A detailed description of the phylogenetic methods and results can be found in the supplementary materials (Supplementary data Method S1, Tables S2-5).

Given that all species with leaf spines and stipular spines were from open habitats and none from closed habitats, we did not have sufficient overlap to conduct multi-linear regressions across all predictors. Therefore, we tested subset combinations of predictors on spine variables. We analysed early spine expression (presence and biomass investment in spines) in two ways. First, spine emergence was treated as a binary response (Yes = 1, No = 0). When examined at week 5 and 15, all individuals of a given species either produced spines or not. Therefore we analysed the spine emergence dataset at the species-level (where a species either produced spines or not). To test predictions 1 and 3 (i.e. the general relationships between sapling size and a species precipitation niche on spine emergence), we implemented independent binomial regression (generalized linear model) models where the presence of spines is predicted by log-transformed sapling size and MAP (i.e. mean annual precipitation under which the species grows in its natural environment). We tested for the interactive effects of sapling mass and spine type (i.e. mass x spine types, prediction 2)) and sapling mass and habitat (i.e. Mass x habitat interaction, prediction 4) on spine emergence by implementing similar binomial models. Where the interaction term was not significant, we implemented additional univariate model testing for

differences in spine emergences for different spine types and habitat type. All the above models were fitted separately for each week (i.e., week 5 and 15) because we were also interested in exploring whether our results changed across these two temporal periods. Binomial models were performed using the “*glm*” function in R version 3.4.4 (R Core Team 2018). For some levels of spine type (e.g. leaf spine at 5 weeks and stipular spines at 15 weeks), all species had produced spines (i.e. spine presence = 1 for all observations). Complete separation (i.e. where a linear combination of predictors is perfectly predictive of the outcome – in our case a level of the factor had only ‘success’ observations – (Gelman *et al.* 2008)) can significantly bias model estimates (Bolker 2015). Thus, for the models testing for differences in spine emergence between species with different spine types, we used the function ‘*bayesglm*’ from the “arm” package (Gelman and Su 2018). The “*bayesglm*” function adopts a Bayesian inference approach and uses minimally informative priors to derive stable regression estimates (Gelman *et al.* 2008; Gelman and Su 2018). We used the default settings for all analyses with the “*bayesglm*” function.

Second, we analysed differences in SPMF (log-transformed) with linear mixed effect models (here, species was treated as a random effect). Similar to the binomial models above, we first tested for the independent effects of sapling mass (measure of size, prediction 1) and MAP (prediction 3) on SPMF. We further ran models testing for sapling mass x spine type (prediction 2) and sapling mass x habitat (prediction 4) effect on SPMF. We fitted all the linear mixed models using the ‘*lmer*’ function implemented in the “lme4” package (Bates *et al.* 2015). All statistical analyses described here were conducted in R version 3.4.4 (R Core Team 2018).

RESULTS

Spine emergence in saplings

At 5 and 15 weeks, spines emerged in 47% (21 of 45) and 69% (31 of 45) of species respectively. Spine emergence was remarkably conserved within species at the two sampling dates. When inspected at 5 or 15 weeks, all individuals of a given species either bore spines or not (except for individuals of *Bombax ceiba* and *Pyracantha fortuneana*).

Binomial models indicated that spine emergence was affected by spine type, species' light and precipitation niche (Table 1). As predicted, probability of spine emergence at week 5 (Fig. 2a; Supplementary data Table S6) was significantly lower in species possessing thorns (19%) compared to species with prickles (54%), stipular spines (83%) and leaf spines (100%, all from the *Berberis* genus). These patterns persisted to week 15. By 15 weeks, all species with stipular spines, 85% of species with prickles and 43% of species with thorns, had produced spines.

In contrast to our prediction, probability of spine emergence was significantly higher in open habitat species relative to closed habitat species at both 5 and 15 weeks (Fig. 2 b, Table 1). Fifty-nine per cent and 80% of species from open habitat, relative to 25% and 50% of species from closed habitat, produced spines at 5 and 15 weeks respectively. Across species (at both 5 and 15 weeks), probability of spine emergence was negatively correlated (Fig. 2c; Table 1) with MAP (i.e. species from higher rainfall environments were less likely to produce spines). We found no associations between spine emergence and sapling size (mass) (Table 1). Further, there was no significant sapling mass x spine types or sapling mass x habitat interaction effect on spine emergence (Table 1). The relationships between spine emergence and the predictor variables were qualitatively similar when analysed at both week 5 and 15 (Table 1).

Biomass allocation to spines in saplings

At 15 weeks, constitutive biomass investment in spines (SPMF) varied from $< 0.1\%$ to 15% of sapling mass across individuals (Mean = 2% ; SD = 2.6%). Expressed as the equivalent of leaf mass (i.e. spine mass as a fraction of leaf mass), sapling investment in spines ranged from 1% to 46% of leaf mass (Mean = 5% ; SD = 7.4%) across individuals.

There was no detectable relationship between saplings mass or precipitation niche on investment in spines (SPMF). However, investment in spines (SPMF) differed by spine type (Table 1) with the highest investment in species with leaf spines (mean $\pm$ SD: $5.2\% \pm 2\%$), followed by species with thorns ($2.5\% \pm 3.5\%$), stipular spines ($1\% \pm 0.9\%$), and the lowest investment in species possessing prickles ($0.7\% \pm 0.9\%$). Importantly, there were significant differences in the trend of biomass allocation to spines (SPMF) with sapling size (mass) for the different spine types (i.e. significant mass x spine type interaction) (Fig.3a; Table 1). Allocation to spines was relatively fixed (no significant change in SPMF with sapling mass) for species possessing stipular spines and leaf spines (Fig.3a). However, SPMF increased for species with prickles but decreased for species possessing thorns (Fig.3a) with increasing sapling mass.

On average, species from open habitat invested ~ 2.3 times more biomass in spines relative to species from closed habitats. The relationship between sapling mass and SPMF was contingent on a species' light niche (i.e. significant sapling mass x habitat interactions). For open habitat species, SPMF was independent of sapling size. On the contrary, SPMF was positively associated with sapling mass for species of closed habitat (Fig. 3b).

DISCUSSION

We have provided the first detailed evaluation of constitutive patterns of early expression of spines among saplings of 45 spinescent species, grown under common conditions. Our approach

has allowed us to explore general patterns in spine expression and to evaluate the influence of sapling size, spine type and species' environmental niche (light and precipitation niche) on early expression of spines. Our results suggest that: 1) the inherent constraints imposed by the developmental pathway of the spine (spine type), and 2) the resource environment (light and rainfall) under which species evolved, are two important factors determining constitutive trajectories of early spine expression. These findings were consistent across the two temporal stages examined, namely weeks 5 and 15 after transplanting. Moreover, the timing of spine emergence, when considered at the two sampling dates, was remarkably conserved within species.

Plant defence theory predicts a tight coupling between growth and defence, particularly under limited resource availability (Coley *et al.* 1985; Herms and Mattson 1992; Stamp 2003). Although this prediction has been largely examined with respect to chemical defences (Bergelson and Purrington 1996; Koricheva 2002; Zuest and Agrawal 2017), for physical defences this relationship was less explored. Here, we tested whether sapling size was related to early emergence and investment in spines. In contrast to our prediction, we showed that sapling size was uncoupled from emergence of spines at both week 5 and 15. This suggests that onset of spines, in early saplings, per se may be unrelated to plant size but rather determined by other factors. While continuous sampling (e.g. weekly) may have yet revealed a link between plant size and spine emergence, the scope of our study characterizing 45 species constrained such an approach. Further studies focusing on fewer species could provide more precise developmental trajectories for sapling growth and spinescence. Although spine emergence was decoupled from sapling size (within the time window assessed), we observed that, across species, total biomass allocation to spines does depend on sapling size, but is contingent upon species light niche and

spine type. Thus, while sapling size is linked to biomass allocation to spines, the nature of this relationship may be mediated by other factors such that a general assessment of growth-defence trade-off across spiny plants, without adequate consideration of the spine type or resource environment, may fail to detect any pattern (Bergelson and Purrington 1996; Koricheva 2002; Moles *et al.* 2013).

Spines types (leaf spine, stipular spine, thorns and prickles) are derived from separate organs or tissues (respectively leaf blades, stipules, branches and epidermis; (Bell and Bryan 2008), and we found that their timing of emergence is strikingly different. Spines derived from leaf parts (leaf spines and stipular spines) and prickles were the earliest to emerge while thorns, which are modified branches, were the last to emerge. Interestingly, we detected no interaction between spine type and sapling size. This means that the slow pace at which thorns emerge across species is not driven by size limitation (independent from either growth rate or absolute internal resource pools). Instead, our results suggest that the developmental sequence in which organs are produced – which follows a typical order in most vascular plants (i.e. organogenesis, growth and branching) (Barthelemy and Caraglio 2007) – exert a strong constraint on the timing of emergence of spines. Thus, the developmental pathway of spines is vital for predicting how fast species will be protected during their most vulnerable early life stages, and the timing of spine emergence reflect anatomical and architectural constraints perhaps more than any other factor (Villamil *et al.* 2013).

We observed divergent patterns of investment in spines for the different spine types across sapling sizes. For leaf and stipular spines, the total biomass of spines was uncoupled from sapling size, suggesting growth and defence may not trade-off in these species. On the contrary, species with prickles invested more in spines and species with thorns invested less as sapling size

increased. It seems unlikely that prickles could be resource limited to incur significant allocation cost (Bazely *et al.* 1991) and therefore respond to sapling size. Kellogg *et al.* (2011) have shown that prickles in *Rubus* follow a 4-stage developmental progression until the final prickle development is completed. Thus, the link between plant size and investment in prickle might be the result of completing these developmental phases earlier in fast growing individuals (and not due to increased resource pools for allocation to spines). The negative relationship between sapling size and investment in thorns (at week 15) possibly points to a trade-off between growth rate (which determines sapling size) and defence allocation (Herms and Mattson 1992; Zuest and Agrawal 2017). Activation and lignification of auxiliary meristems to produce thorns potentially consumes more resources and incurs higher allocation costs in saplings than other types of spines. Here our results imply that regulation and expression of spines differ for the different spine types and therefore lumping these traits generally as ‘spinescence’ (see for example: Grubb 1992; Hanley *et al.* 2007; Moles *et al.* 2013) potentially limit our ability to predict their ecological performance.

Our analysis suggests that early spine expression across species is influenced by a species’ native resource environment (precipitation and light niche). We found a significant negative relationship between precipitation and spine emergence (Fig. 2c). This result supports the prediction that species from resource limited environments (here, low precipitation) are selected for greater constitutive defence expression (Coley *et al.* 1985; Swihart and Bryant 2001). In low precipitation environments, both photosynthesis and growth are constrained by water limitation (e.g. during dry season). Here, plants cannot grow fast to reach heights that would allow them to quickly escape ground-based herbivores (Grubb 1992) and thus must invest more in defences (Coley *et al.* 1985; Herms and Mattson 1992). In contrast, emergence and

investment in spines was greater in species from open than those from closed habitats (Fig.2b and Fig. 3b). These results partially contradict the hypothesis that defence is higher in species from low resource environments (here species from closed habitat: Coley *et al.* 1985). It may be energetically expensive for saplings (e.g. due to low light supply) to allocate limited carbohydrates to spines under closed environments. Consistent with this notion, we observed increased allocation to spines with increasing sapling size for species from closed habitats (Fig.3b) suggesting that spiny species adapted to closed habitats may allocate more resources in defence when sufficient mass has been reached(Grubb 1992; Swihart and Bryant 2001).

Beyond the relevance of this work, our results should be considered with caution for two main reasons. Firstly, species were grown under a single common garden (i.e. unnatural conditions for most species) and thus patterns of spine expression may be obscured for some species (Poorter *et al.* 2016). Determining the right conditions under which to examine early spine expression, for multiple species sourced from contrasted environments, is quite difficult, and the advantage of common garden studies is that they allow for comparisons across phylogenetically diverse plants under highly controlled and similar environmental conditions. Previous studies examining resource supply on spinescence have so far produce inconsistent results (Hanley *et al.* 2007) and thus failed to provide clear patterns on how fertilization, light, and water affect spine expression. For instance, fertilization has been reported to have positive (Gowda *et al.* 2003), negative (Bazely *et al.* 1991), or no (Pisani and Distel 1998; Cash and Fulbright 2005) effect on spine expression. In turn, shading has had no (Bazely *et al.* 1991) or positive (Barton 2014) effects on spine production, whereas irrigation has no detectable influence on spine production (Pisani and Distel 1998; Barton 2014). Hence, deciding what environmental conditions are best for testing this question is still a challenge.

Secondly, given that spines have most likely evolved as defence mechanisms against large herbivores (Hanley *et al.* 2007; Charles-Dominique *et al.* 2016), constitutive patterns of early spine expression are likely to reflect historical patterns of large mammalian herbivory (McKey 1974; Rhoades 1979). However, attributing patterns of spine expression observed in this study to large herbivory pressure (or using a proxy) is difficult given the existing data on mammalian herbivores. The majority of our species are native to China and Southeast Asia and have ranges that previously contained significant concentrations of large herbivores (wild cattle, deer, rhinoceros, and elephant) similar to the fauna of Africa (Harris *et al.* 1953; Wharton 1966; Biasatti *et al.* 2012; Corlett 2014). However, the composition and distribution of large mammalian herbivores in Southeast Asia have changed remarkably in recent times, and in most habitats, large herbivores have been extirpated (Corlett 2007), making it difficult to attribute variations in herbivory pressure to the observed animal densities. Further, some spine types (such as prickles and leaf spines) could also have evolved in response to invertebrate herbivores (Kariyat *et al.* 2017). Thus attributing constitutive patterns of spine expression to a specific class of herbivore requires an understanding of which class of herbivore is specifically targeted by these different spine types.

In spite of the potential limitations of inferring evolutionary sources of species variation in common garden studies, our approach has elucidated some of the general trends in the timing and investment in spines during early plant growth. Indeed our results are consistent with patterns observed for other plant defence types. For instance, similar to our results, there is increasing evidence that expression of chemical (e.g. polyphenols and monoterpenoid: Murray and Hackett 1991; Goralka and Langenheim 1996; Fernandez-Lorenzo *et al.* 1999), physical (e.g. toughness: Rafferty and Lamont 2007; Kitajima *et al.* 2013; Mason and Donovan 2015) and

indirect defences (Brouat and McKey 2000; Villamil *et al.* 2013) are limited during early ontogeny because key features and pathways are not well developed in juveniles (Barton and Hanley 2013; Barton and Boege 2017). Further, while the incidence of sclerophylly, tough leaves (i.e. high LMA) and pubescens (trichomes) is higher in resource limited environments (e.g. low precipitation and fertility: Coley *et al.* 1985; Hanley *et al.* 2007), these physical defence traits respond positively to high light intensities (Groom and Lamont 1997; Roberts and Paul 2006; Hanley *et al.* 2007). Similarly, chemical defences are thought to be greater in species from resource limited environments (Coley *et al.* 1985; Stamp 2003), yet there is substantial evidence that species growing under high light conditions have greater concentrations of both carbon and non carbon-based secondary metabolites than those growing under shade (Koricheva *et al.* 1998; Roberts and Paul 2006).

CONCLUSION

We conducted the first study examining the pattern of emergence and investment in spines in woody saplings across a diverse species pool. Generally, our results suggest that variation in the onset of spines depends on the spine type possessed by a species and the environment from which species originate (i.e. light and precipitation niche). Further, we found that, across species, investment in spine was influenced by sapling size but this effect was contingent on spine type and light niche. Due to the striking differences in early spine expression observed in this study, we advocate that understanding effectiveness of the different spine types against large herbivores is an important next-step in developing predictive frameworks on how herbivory and environmental resources – and potential changes in these – will shape spiny woody communities in the future. Our study, focusing on structural defence in woody juveniles (most of which are

tropical species) complements similar work from temperate and boreal forests which mostly focused on chemical defences. Our findings, together with other studies, provides insights into the roles of herbivory, environment and developmental controls on defence investment in juvenile woody plants. Finally, we suggest that understanding how resource limitation and herbivory interact to influence developmental shifts in different spine types could improve predictions for how future changes in resource supply and herbivore abundances will influence spiny plant communities.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following.

Method S1: Details of phylogenetic methods used in this study. **Table S1:** Summary information on the 45 species used in this study. **Table S2:** Genbank accession numbers for DNA sequences of the 45 species used in this study. **Table S3:** Model summary of phylogenetically adjusted model testing for factors affecting spine emergence. **Table S4:** Summary of pairwise comparison on probability of spine emergence between spine types based of phylogenetically adjusted model. **Table S5:** Model summary of phylogenetically–adjusted models testing for effect of factors on biomass investment in spines. **Table S6:** Model summary of pairwise comparison on probability of spine emergence between spine types based on ordinary binomial model.

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Table 1: Results of generalized linear (glm) and mixed effect (LMMs) models testing the effects of plant size, mean annual precipitation, habitat and spine type on probability of emergence and biomass investment in spines in saplings of 45 species grown under greenhouse conditions. Significant effects (i.e. $p < 0.05$) were assessed with likelihood ratio test and are highlighted in bold. Spine emergence data was assessed 5 and 15 weeks after transplanting seedlings to common garden whereas spine mass fraction (SPMF) was determined at week 15 only.

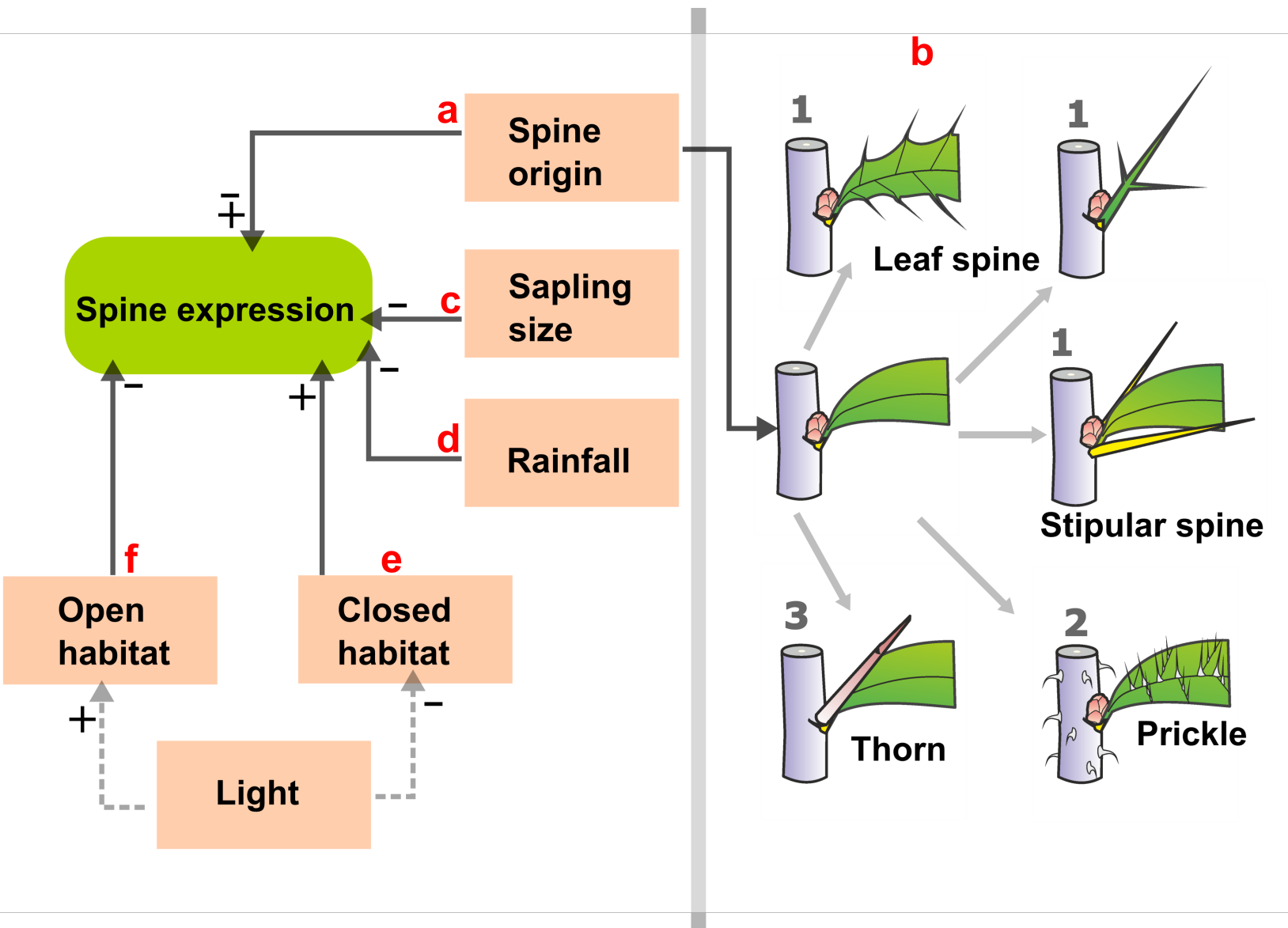
Model	d.f	Spine emergence (Week 5)		Spine emergence (Week 15)		Spine Investment (SPMF, Week 15)	
		LRT	<i>p</i> -value	LRT	<i>p</i> -value	LRT	<i>p</i> -value
log(mass)	1	0.634	0.426	0.009	0.921	2.369	0.114
MAP	1	9.519	0.002	9.937	0.001	1.863	0.173
Spine type	3	16.762	<0.001	19.241	<0.001	9.529	0.023
Habitat	1	4.852	0.028	4.746	0.029	2.197	0.138
Log (mass) * Spine type							
log(mass)	1	0.188	0.980	0.000	1.000	0.00	-
Spine type	3	4.708	0.319	4.844	0.184	20.691	<0.001
log(mass)* spine type	3	0.727	0.948	2.181	0.536	26.793	<0.001
Log(mass) * Habitat							
Log(mass)	1	0.113	0.74	1.591	0.207	0.00	-
Habitat	1	4.199	0.04	5.767	0.016	6.060	0.014
Log(mass)* habitat	1	0.308	0.58	1.622	0.203	7.575	0.006

FIGURE LEGENDS

Figure 1. Conceptual diagram showing how different factors are predicted to affect early spine expression in saplings. Symbols at end of arrows indicate positive effect (+), negative (-) or positive and negative effects (+/-). Solid and broken lines represent predictions that were tested and implied but not directly tested in this study, respectively.

Figure 2. Probability of spine emergence after 5 weeks A) across spine types, B) between open and closed habitats, C) along precipitation preference gradient. For both A) and B) circles represent mean probability and error bars are 95% confidence intervals for the means. For C), solid line represents mean predicted probability and dashed grey lines show the 95% confidence intervals. For A and B, differences in spine emergence were assessed using multiple comparison tests (Tukey).

Figure 3. Relationship between sapling mass and biomass investment (as a percentage of total sapling mass) for species A) possessing different spine types and B) from different habitats grown under common garden conditions for 15 weeks.



Probability of producing spines

