

Geophysical, evolutionary and ecological processes interact to drive phylogenetic dispersion in angiosperm assemblages along the longest elevational gradient in the world

HONG QIAN^{1,*}, BRODY SANDEL^{2,†}, TAO DENG^{3,*} and OLE R. VETAAS⁴

¹Research and Collections Center, Illinois State Museum, 1011 East Ash Street, Springfield, IL 62703, USA

²Department of Biology, Santa Clara University, 500 El Camino Real, Santa Clara, CA 95057, USA

³CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

⁴Department of Geography, University of Bergen, Fosswinckels gt. 6, PO Box 7802, N-5020 Bergen, Norway

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Ecologists have embraced phylogenetic measures of assemblage structure, in large part for the promise of better mechanistic inferences. However, phylogenetic structure is driven by a wide array of factors from local biotic interactions to biogeographical history, complicating the mechanistic interpretation of a pattern. This may be particularly problematic along elevational gradients, where rapidly changing physical and biological conditions overlap with geological and biogeographical history, potentially producing complex patterns of phylogenetic dispersion (relatedness). We focus on the longest elevational gradient of vegetation in the world (i.e. c. 6000 m in Nepal) to explore patterns of phylogenetic dispersion for angiosperms (flowering plants) along this elevational gradient. We used the net relatedness index to quantify phylogenetic dispersion for each elevational band of 100 m. We found a zig-zag pattern of phylogenetic dispersion along this elevational gradient. With increasing elevation, the phylogenetic relatedness of species decreased for the elevational segment between 0 and c. 2100 m, increased for the elevational segment between 2100 and c. 4200 m, and decreased for the elevational segment above c. 4200 m. We consider this pattern to be a result of the interaction of geophysical (e.g. plate tectonics) and eco-evolutionary processes (e.g. niche conservatism and trait convergence). We speculate on the mechanisms that might have generated this zig-zag pattern of phylogenetic dispersion.

ADDITIONAL KEYWORDS: angiosperms – environmental gradient – Himalayas – phylogenetic relatedness.

INTRODUCTION

There has been a recent explosion of interest in using patterns of phylogenetic relationships to gain ecological and evolutionary insights. Many studies take a ‘phylogenetic-patterns-as-proxy’ approach (Gerhold *et al.*, 2015), in which the phylogenetic pattern is interpreted as evidence for the action of some ecological or evolutionary mechanism. This approach was first applied in community ecology, typically with the goal of discerning the relative

influence of environmental filtering and competition (Webb, 2000; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004). In local communities, environmental filtering is expected to lead to the success of certain clades and failure of others, causing the favoured clades to be over-represented and therefore phylogenetic clustering to be high and phylogenetic diversity to be low. On the other hand, if competition is most intense between closely related species, strong competition should lead to relatively high phylogenetic dispersion and diversity (Webb, 2000; Webb *et al.*, 2002).

This simple interpretation is complicated by the fact that other factors, such as biogeographical and evolutionary history, can also drive patterns of phylogenetic structure. For example, the ‘tropical

*Corresponding authors. E-mail: dengtao@mail.kib.ac.cn; hqian@museum.state.il.us

†These authors contributed equally to this work.

niche conservatism' (TNC) hypothesis (Wiens & Donoghue, 2004) posits that when tropical lineages withdrew from high latitudes with post-Eocene global climate cooling, some lineages acquired adaptations to temperate climates. These few lineages then diversified in the new cooler environments, forming clusters of closely related species. As a result, colder regions should contain more closely related species, increasing phylogenetic clustering and reducing phylogenetic diversity (Hawkins *et al.*, 2006; Buckley *et al.*, 2010; Qian *et al.*, 2013, 2016). Angiosperms (flowering plants) originated and diversified during the Mesozoic with a globally warm climate, and traits that enable angiosperms to tolerate low temperature have generally evolved since post-Eocene global climate cooling (Takhtajan, 1969). Empirical data from latitudinal gradients are generally consistent with this prediction (Qian *et al.*, 2013, 2016). If phylogenetic dispersion is primarily driven by temperature, one would expect that the same trend should be observed for elevational gradients.

Several studies have investigated patterns of phylogenetic dispersion (relatedness) of plants along elevational gradients, but results are mixed. For example, angiosperm species tend to be more closely related to each other (i.e. show stronger phylogenetic clustering) at higher elevations in a temperate region in Asia (Qian, Hao & Zhang, 2014), which is consistent with the TNC hypothesis. In contrast, angiosperm tree species tend to be more distantly related to each other (i.e. show stronger phylogenetic over-dispersion) at higher elevations in tropical regions in Asia and South America (Culmsee & Leuschner, 2013; Qian & Ricklefs, 2016). Because previous studies on the phylogenetic dispersion of plants for elevational gradients used relatively short elevational gradients and did not include alpine zones, each of these studies observed only part of the pattern of phylogenetic dispersion that may exist along a complete elevational gradient. Furthermore, because most previous studies on the phylogenetic dispersion of angiosperms for elevational gradients included only tree species, which typically account for a small proportion of species in an entire angiosperm flora, these studies might have missed true patterns of phylogenetic dispersion of angiosperms along elevational gradients.

The elevational gradient of the central Himalayas in Nepal is the longest in the world, ranging from 60 to 8848 m above sea level (a.s.l.), >6000 m of which is inhabited by angiosperms. The mixed results of previous studies lead us to predict different patterns, depending on the elevational zone considered.

The elevational gradient of Nepal represents a long temperature gradient from subtropical to alpine climate. We predict that the phylogenetic relatedness

of angiosperms increases with elevation across the overall elevational gradient of Nepal (hypothesis 1), as predicted by the TNC hypothesis.

However, low elevations in Nepal include lands from both the Eurasian plate (part of Laurasia) and the Indian plate (part of Gondwana), which had floras with different evolutionary histories before the collision of the Indian plate into the Eurasian plate *c.* 50 Mya. This collision resulted in an uplift of several thousand metres in Nepal and other part of the Himalayas. The boundary (fault line) between the two plates is located in Nepal (Jin & McNutt, 1996). Thus, the flora of Nepal is a mixture of the palaeofloras of Laurasia and Gondwana. Combining clades from different biogeographical regions is expected to decrease phylogenetic clustering because clades from different biogeographical regions are expected to be more widely spread across the phylogenetic tree than those from a single biogeographical region (Sandel & Tsirogiannis, 2016). Because the Indian plate is expected to have contributed more species and higher level taxa (e.g. families and orders) to lower elevations (due to more area from the Indian plate at lower elevations), compared with higher elevations, and because species in the Indian plate were probably phylogenetically clustered, relative to the combination of the Indian and Asian floras, a greater degree of phylogenetic clustering is expected at a lower elevation, at least in the elevational segment with lowlands in Nepal. In addition, the 'out of tropical lowlands' hypothesis, which was proposed based on niche convergence to explain the patterns of increasing mean family age and decreasing phylogenetic relatedness with increasing elevation observed for angiosperm trees in tropical regions (Qian & Ricklefs, 2016), might also have played a role in generating the patterns of phylogenetic dispersion at lower elevations in Nepal. Accordingly, we predict that the phylogenetic relatedness of angiosperms decreases with increasing elevation in a segment with relatively low elevations in Nepal (hypothesis 2).

Above the treeline, which occurs at *c.* 4000–4200 m in Nepal, the microclimatic and physical conditions experienced by plants change dramatically. Among these changes are an increase in exposure to UV radiation (Turunen & Latola, 2005), which can be an important stress for plants (Jansen, Gaba & Greenberg, 1998). Herbaceous lineages adapted to the cold, shady environments below the treeline might perform poorly under full UV exposure above it, suggesting that there might be a restructuring of phylogenetic composition around the treeline. To the extent that the potential to evolve UV tolerance, in addition to tolerance for the other conditions that make the alpine zone distinct, is phylogenetically conserved, this should strengthen

the pattern of increasing phylogenetic clustering with greater elevation in the alpine zone (hypothesis 3). A previous study has indeed demonstrated that plants in the alpine zone of the Hengduan Mountains are phylogenetically more closely related with increasing elevation (Li *et al.*, 2014).

The goal of this study was to test for the above-outlined three hypotheses: that the phylogenetic relatedness of angiosperms (1) generally increases with elevation across the overall elevational gradient of Nepal, (2) decreases with elevation at lowlands and (3) shows a shift near the treeline, but probably continues to increase with elevation.

MATERIAL AND METHODS

STUDY AREA

The Himalayan range that falls within Nepal is located between 80°04'–88°12'E and 26°22'–30°27'N, with an elevational gradient ranging from 60 to 8848 m a.s.l. According to van Hinsbergen (2012), the area where the Himalayas in general and Nepal in particular are located was part of the Indian plate, which was in turn part of Gondwana and remained connected to other Gondwanan plates until at least 140 Mya. The Tibetan–Himalaya microcontinent collided with the Asian plate *c.* 50 Mya (i.e. the 'soft' India–Asia collision). The 'hard' India–Asia collision occurred *c.* 25–20 Mya. Thus, Nepal has been connected to the Asian plate since 50 Mya and has been re-connected to the Indian plate since *c.* 25 Mya (for a complete reconstruction of tectonics in the region, see van Hinsbergen, 2012). Before the hard collision, elevation in the region with Nepal ranged from sea level to about 5000 m (Ding *et al.*, 2014; Deng & Ding, 2015). During the Eocene and Oligocene, areas of low elevation in the region remained covered by tropical/subtropical forests with tree species closely related to those that are distributed in present-day typical subtropical broad-leaved forests in Asia (Axelrod, Al-Shehbaz & Raven, 1998).

FLORISTIC DATA

We obtained species lists and elevation ranges of angiosperms in Nepal from the *Enumeration of flowering plants in Nepal* (Hara, Stearn & Williams, 1978; Hara & Williams, 1979; Hara, Chater & Williams, 1982) and the *Annotated checklist of the flowering plants of Nepal* (Press, Shrestha & Sutton, 2000; available at http://www.efloras.org/flora_page.aspx?flora_id=110). [We did not include non-angiosperms (pteridophytes and gymnosperms) in this study because they have different evolutionary histories than angiosperms (Hawkins *et al.*, 2014).] We standardized the botanical

nomenclature of the Nepalese angiosperms according to The Plant List (version 1.1; <http://www.theplantlist.org/>). Intraspecific taxa were combined at the species level. Non-native species were excluded. The final data set included 4290 species of angiosperms, belonging to 1327 genera and 185 families.

We divided the entire elevation gradient of Nepal into 89 100-m vertical bands with the lowest band located between 0 and 100 m a.s.l. Following previous studies (e.g. Patterson, Pacheco & Solari, 1996; Rahbek, 1997; Fleishman, Austin & Weiss, 1998; Brühl, Mohamed & Linsenmair, 1999; Odland & Birks, 1999; Vetaas & Grytnes, 2002), a species was assigned to all vertical bands which were entirely or partially located within the elevation range of the species. Some species might not occur in every vertical band within their elevational ranges. However, our goal was not to quantify the phylogenetic structure of actual assemblages, but rather to describe the phylogenetic structure of species capable of surviving at a particular elevation. This information is conveyed by the elevational bands, even if actual co-occurrence is not. The first 65 vertical bands of the Nepalese elevation gradient were occupied by at least one angiosperm species. The two vertical bands below 200 m a.s.l. each contained <200 species and the five vertical bands above 6000 m a.s.l. each contained <10 species; we excluded these bands in data analysis to minimize the issue of under-sampling. As a result, the elevational gradient of this study included 58 vertical bands ranging from 200 to 6000 m. Of the vertical bands included in this study, none at elevations of <4600 m had <450 species and none at high elevations had <50 species except for the three vertical bands at elevations of >5700 m.

PHYLOGENETIC RECONSTRUCTION AND PHYLOGENETIC METRICS

We used the mega-phylogeny 'Phytophylo' (Qian & Jin, 2016; available at <https://github.com/jinyizju/>) as a backbone to generate a phylogenetic tree for the Nepalese species. Phytophylo is an updated version of the mega-phylogeny published by Zanne *et al.* (2014). The relationships among all angiosperm families in the world have been completely resolved in Phytophylo. Of the 1327 angiosperm genera in Nepal, 1086 (81.8%) are included in Phytophylo. For those genera and species in our data set that are absent from Phytophylo, we used the software S.PhyloMaker (Qian & Jin, 2016; available at <https://github.com/jinyizju/>) to add them to their respective families (in the case of genera) and genera (in the case of species) in the mega-phylogeny using Scenario 3, which is analogous to using Phylomatic with Bladj to generate a phylogenetic tree (Webb, Ackerly & Kembel, 2008). The method that we used to generate a phylogenetic

tree for plants is broadly used in the literature (e.g. Carvajal-Endara *et al.*, 2017; Freschet *et al.*, 2017; Qian & Sandel, 2017). We pruned the mega-phylogeny to include only species present in Nepal.

We used the net relatedness index (NRI) as a measure of phylogenetic dispersion of species assemblages within each elevation band. NRI is defined as (Webb, 2000):

$$\text{NRI} = -1 \times (\text{MPD}_{\text{observed}} - \text{MPD}_{\text{randomized}}) / (\text{sdMPD}_{\text{randomized}}), \quad 1$$

where $\text{MPD}_{\text{observed}}$ is the observed MPD (mean phylogenetic distance), $\text{MPD}_{\text{randomized}}$ is the expected MPD of the randomized assemblages and $\text{sdMPD}_{\text{randomized}}$ is the standard deviation of the MPD for the randomized assemblages. We chose to use NRI over other phylogenetic metrics for two reasons. First, NRI is calculated based on the standardized effect size, which accounts for differences in species richness among the assemblages concerned, describes the average relatedness of a pair of species in a sample (Webb *et al.*, 2002; Webb, Ackerly & Kembel, 2011), is the most widely used phylogenetic structure metric, and is used in the vast majority of previous studies on phylogenetic relatedness for elevational gradients. Thus, using NRI in this study will make the results directly comparable with those of other studies. Second, because NRI is based on the mean pairwise distance among all species in the sample across the tree, rather than weighting basal or tip divergences more heavily, it is particularly suited to measure phylogenetic relatedness resulting from deep (ancient) and shallow (recent) evolutionary events. NRI is standardized to the mean and expectation of the mean pairwise distance given the tree and species richness of the sample. A positive NRI value indicates phylogenetic clustering, whereas a negative value indicates phylogenetic overdispersion. We used the software PhyloMeasures (Tsirogiannis & Sandel, 2016) to calculate NRI. With PhyloMeasures, NRI was computed using computationally efficient algorithms described in Tsirogiannis, Sandel & Cheliotis (2012) and Tsirogiannis, Sandel & Kalvisa (2014). Specifically, PhyloMeasures calculates NRI not based on a resampling approximation of the mean and variance, but rather based on exact solutions given a particular phylogenetic tree and species richness. Our null model considers all possible combinations of S species from the species pool (where S is the richness of a sample to be standardized) to be equally likely (Tsirogiannis & Sandel, 2016). We used all angiosperm species of Nepal as a species pool when calculating each value of NRI.

To demonstrate that variation in species richness among sampling units would have no effect on our results, we conducted a rarefaction analysis. Specifically, we randomly sampled 100 species from

each of the 100-m bands that have sufficient species to generate robust assemblages with 100 species (i.e. all bands between 200 and 5000 m in elevation) and calculated NRI; we repeated this 1000 times and calculated a mean value for each band. If the correlation between NRI derived from all species in a band and NRI derived from resampling of 100 species in a band is positive and strong, we conclude that variation in species richness among elevational bands would have no effect on the conclusion of this study.

Supplementing this main analysis, we also conducted analyses assessing uncertainty in NRI values arising from uncertainties in the source phylogenetic tree. We repeated all NRI calculations for each of 100 bootstrap replicate trees provided by Zanne *et al.* (2014). In this case, we reduced our data to the genus level, because the match between our species list and that on the phylogenetic trees was poor, although we took into account the species richness of each genus (i.e. calculating abundance-weighted NRI). In addition, we explored patterns of another phylogenetic structure metric, the phylogenetic diversity index (PDI), which is the standardized effect size of phylogenetic diversity (Tsirogiannis & Sandel, 2016). Analogous to NRI, PDI is the richness-standardized version of Faith's (1992) phylogenetic diversity metric (PD). Unlike NRI, high PDI values indicate high phylogenetic diversity, or low phylogenetic relatedness. We computed PDI both for the species-level data set using Phytophylo and for the genus-level data set across 100 trees from Zanne *et al.* (2014), as for NRI.

CLIMATIC DATA

Mean annual temperature (BIO1), annual precipitation (BIO12), temperature seasonality (BIO4) and precipitation seasonality (BIO15) are among the most important climate variables that shape macroecological and phylogenetic patterns (e.g. Kamilar, Beaudrot & Reed, 2015; Weigelt *et al.*, 2015; Patrick & Stevens 2016). Accordingly, we related NRI to these climate variables (minimum temperature of the coldest month is also a commonly used climate variable but it is strongly correlated with mean annual temperature for elevational bands in our data set; $r = 0.996$). We obtained data for these climate variables from the WorldClim database (Hijmans *et al.*, 2005; <http://www.worldclim.org>). The mean value of each of the four climate variables was calculated for each vertical band using all 30-arc-second resolution data points within the vertical band (on average, 2862 data points for each 100-m elevational band). Using averaged climate data from the WorldClim database to characterize climate conditions for each 100-m elevational band is common in ecological and biogeographical studies, including

those for the Nepalese elevational gradient (e.g. Feng *et al.*, 2016). Correlation among the four climate variables are shown in the Supporting Information (Table S1). In addition to using WorldClim climate data for the main analyses of this study, we also used CHELSA climate data (<http://chelsa-climate.org/>) for a supplementary analysis, so that the results based on the two climate data sources can be compared.

DATA ANALYSIS

We plotted values of phylogenetic metrics against elevation and climate variables, and used correlation analysis to determine the relationships between phylogenetic metrics and climate variables. Because elevational bands are spatially autocorrelated, we did not use the *P*-value to assess the strength of a correlation. Instead, we assessed the strength of a correlation based on the correlation coefficient (weak for $r \leq 0.33$, intermediate for $0.33 < r \leq 0.66$, strong for $r > 0.66$).

RESULTS

The angiosperm flora of Nepal is rich, comprising >4000 species. In general, the NRI of angiosperms tends to increase with elevation in Nepal (Fig. 1). However, it exhibits a triphasic zig-zag pattern: it decreases with increasing elevation up to c. 2000 m a.s.l., increases with increasing elevation from c. 2000 to c. 4200 m and decreases again with increasing elevation from c. 4200 m (Fig. 1). The zig-zag pattern of phylogenetic dispersion was also apparent when the data were analysed at the genus level (i.e. at a deeper evolutionary level), except that the pattern for the elevational segment of c. 4200 m was more variable at the genus level than at the species level (Supporting Information, Fig. S1). The correlation between NRI based on all species in a band and NRI based on resampling 100 species in a band was strong ($r = 0.943$), indicating that variation in species richness among elevational bands did not affect our results.

Patterns of phylogenetic structure measured by PDI were largely congruent with those of NRI (Supporting Information, Figs S1, S2). PDI decreased strongly across the mid-elevations and increased above c. 4000 m. However, patterns in the lowest elevational segment were less clear.

Each of the four climate variables was strongly correlated with NRI in each of the three elevational segments, except for annual precipitation at elevations of <2000 m, but signs (directions) of correlations were opposite among the three elevational segments (Figs 2, 3). For example, mean annual temperature was strongly and positively correlated with NRI in the

low- and high-elevational segments ($r = 0.884$ and 0.937 , respectively), but was strongly and negatively correlated with NRI in the mid-elevational segment ($r = -0.961$; Fig. 3). However, note that these segments were defined a posteriori by dividing the triphasic NRI patterns, and therefore these correlations are maximized. Thus, of the three elevational segments in Nepal, only the pattern of phylogenetic relatedness in the middle segment of the elevational gradient is consistent with the TNC hypothesis. In general, the phylogenetic pattern along the entire elevational gradient of Nepal cannot be consistently explained by any current climatic variables examined (Figs 2, 3).

Climate data extracted from the WorldClim data set correlated strongly with those extracted from the CHELSA data set for three of the four climate variables examined ($r = 0.890$ – 0.998 for BIO1, BIO12 and BIO15) and moderately for the other variable ($r = 0.583$ for BIO4). The relationships between NRI and the climate variables within individual elevational segments were generally consistent between the two climate data sources except for BIO4 and BIO12 in the lowest elevational segment and BIO15 in the highest elevational segment (compare Fig. 3 with Supporting Information, Fig. S3).

DISCUSSION

We used two metrics (i.e. NRI and PDI) to examine patterns of phylogenetic dispersion for angiosperm assemblages distributed across the Nepalese elevational gradient. Both metrics measure phylogenetic dispersion as the standardized effect size, which accounts for differences in species richness (Kerckhoff, Moriarty & Weiser, 2014; Mazel *et al.*, 2016). Patterns derived from NRI and PDI are generally congruent. Slight differences in patterns derived probably emerged because the two metrics highlight phylogenetic structure of assemblages at different evolutionary depths: NRI captures more basal (deeper) structure in a phylogenetic tree, whereas PDI captures more terminal (tip-level) structure (Mazel *et al.*, 2016). Because our goal was to investigate patterns of phylogenetic dispersion across the whole phylogenetic tree (i.e. mean phylogenetic distance of species in an assemblage), rather than focusing on tips of the tree, and because tip-based metrics are more sensitive to low phylogenetic resolution at tips in a phylogeny, compared to NRI (Mazel *et al.*, 2016), we discuss the results derived from NRI below.

We observed a complex elevational pattern of NRI. For a substantial portion of the elevational gradient (between 2000 and 4200 m) the pattern of increasing phylogenetic relatedness with elevation is consistent with hypothesis 1. However, there were negative

relationships between phylogenetic relatedness of angiosperm species and elevation at the lowest and highest elevations. The pattern at low elevations was consistent with hypothesis 2, whereas the transition observed near the treeline was predicted by hypothesis 3, but the ensuing decrease in phylogenetic relatedness with elevation was not.

We consider the zig-zag pattern of phylogenetic dispersion observed in this study to be a result of the interaction of geophysical (e.g. plate tectonics) and eco-evolutionary processes (e.g. niche conservatism and trait convergence). Although phylogenetic relatedness is negatively correlated with elevation in both low and high segments of the Nepalese elevational gradient (i.e. decreasing phylogenetic clustering with increasing elevation), we believe that the same pattern has been driven by different mechanisms, which we discuss below. Because it remains unclear what mechanisms have generated this zig-zag pattern of phylogenetic dispersion for angiosperms across the Nepalese elevational gradient, the mechanisms that are proposed below remain speculative until they are tested when appropriate data are available.

THE LOW ELEVATIONAL SEGMENT (60–2100 M)

Several mechanisms may be involved in the trend of decreasing phylogenetic relatedness with increasing elevation within the elevational segment from 60 m to c. 2100 m in Nepal (Fig. 1). First, because the land currently occurring at elevations below c. 2000 m before the India–Asia collision was uplifted to elevations above c. 2000 m, the entire elevation gradient currently below 2100 m was newly uplifted, resulting from the thrusting of India beneath Asia. Elevations near the upper end of this segment of the Nepalese elevational gradient were once at or near sea level at the time when India initially collided with Asia. After the India–Asia collision, Gondwanan plants carried by the Indian plate mixed with Laurasian plants that evolved on the Asian plate (Raven & Axelrod, 1974). Because these two tectonic plates were separated for tens of millions of years before the collision (Briggs, 1987), their angiosperm evolutionary histories differed substantially, and each plate had a unique set of clades (Raven & Axelrod, 1974). When the two plates collided, major clades (e.g. orders or families) in the mixed flora would probably have been more widely distributed across the angiosperm phylogeny, compared to the plant assemblage of either the Indian plate or the Asian plate. Although some clades in an area might have been filtered out while the area was gradually uplifted to colder elevations due to the Indian plate progressively under-thrusting the Asian plate, clades from both Laurasia and Gondwana might have evolved some genera and species that could adapt to cold

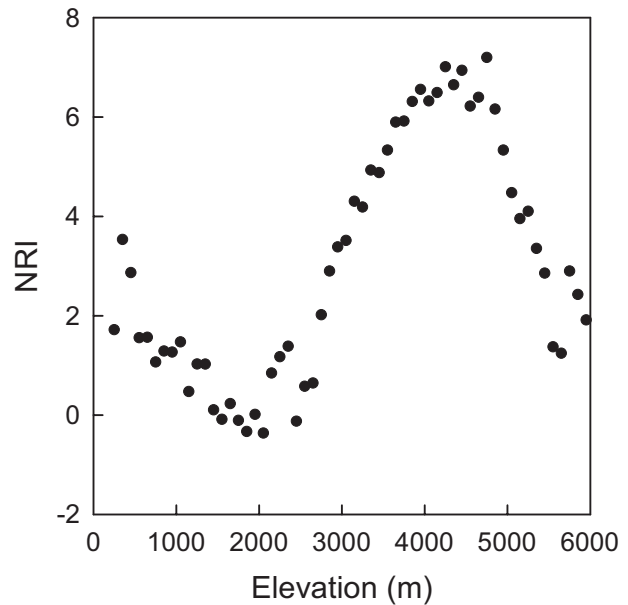


Figure 1. Relationship between net relatedness index (NRI) and elevation for angiosperm species in Nepal.

climates. As a result, species in an assemblage would have remained phylogenetically over-dispersed even when the area with the plant assemblage was uplifted from a low elevation ultimately to c. 2000 m or higher.

In contrast, a larger proportion of plant species from Gondwanan angiosperm clades carried with the Indian plate might have become members of plant assemblages at lower elevations in the segment of the elevational gradient below c. 2000 m. This is because the current low elevations (particularly <500 m) in Nepal are located at or near the boundary between the Indian plate and the Asian plate (or the Tibetan–Himalaya microcontinent) (van Hinsbergen *et al.*, 2012), whereas plants that occurred at low elevations before the India–Asia collision were uplifted to higher elevations. Subsequently, continuous uplift of the Himalayas produced a greater dispersal barrier for newly evolved plant species of Asia. This would have resulted in a greater degree of phylogenetic clustering at a lower elevation within the elevational segment of 60–2100 m because the Indian plate contributed more species to lower elevations and because species of the Indian plate were probably phylogenetically clustered, relative to the combination of the Indian and Asian floras. The latter flora had a much larger proportion of genera and species evolving from the Asian palaeoflora. Although many Gondwanan plant clades initially on the Indian plate went extinct due to climate change after it broke from Africa and travelled for >100 Myr before it collided with Asia (Raven & Axelrod, 1974), some angiosperm families originally on the Indian plate do survive (Raven & Axelrod, 1974).

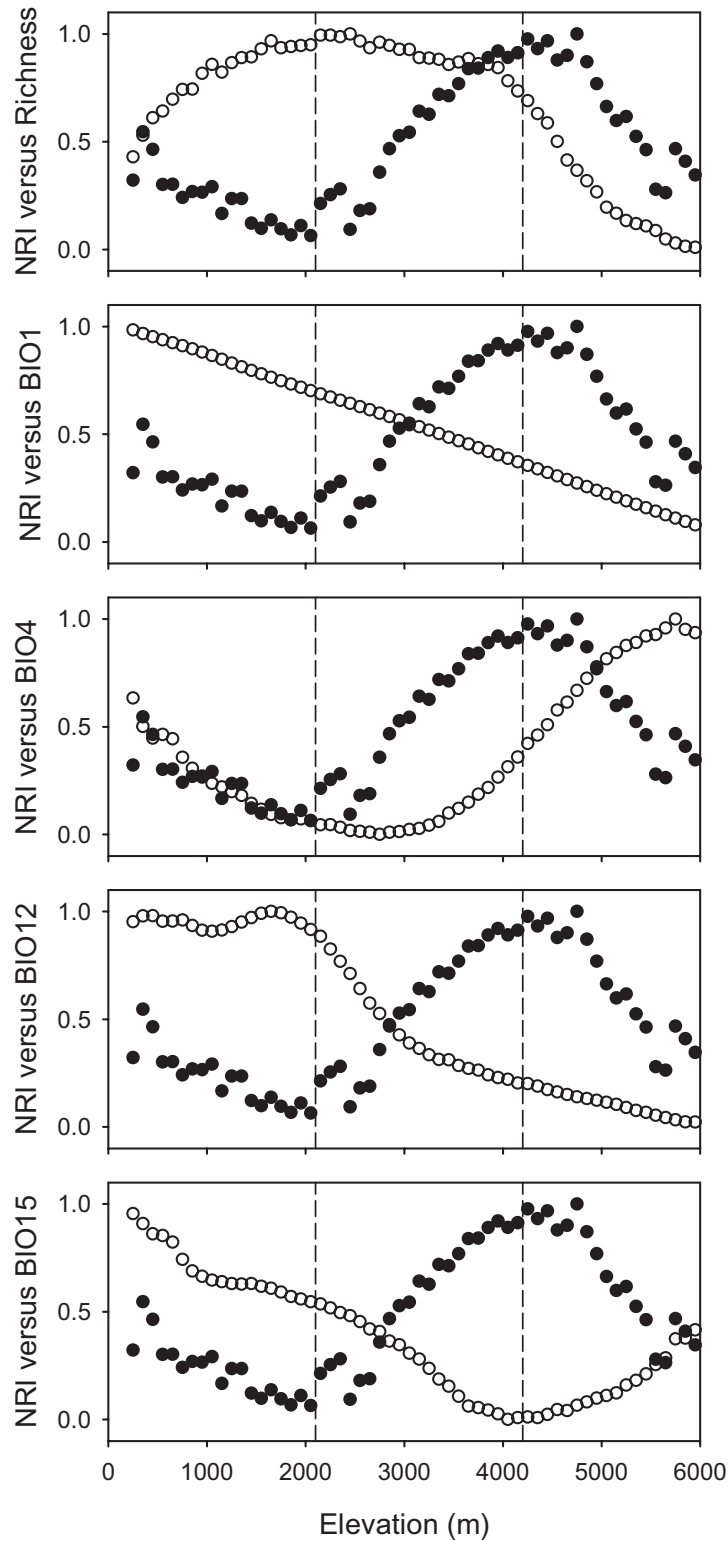
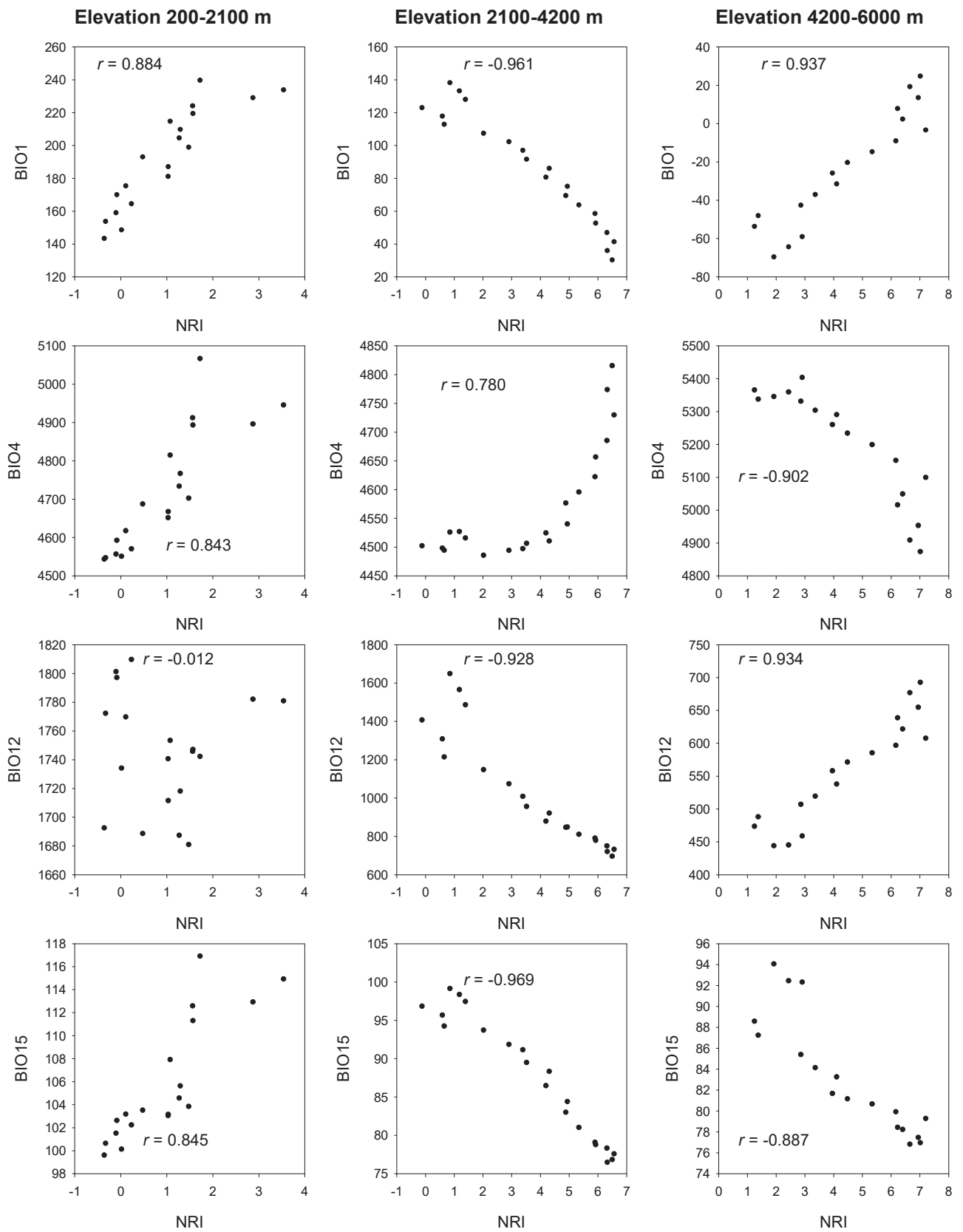


Figure 2. Relationship between net relatedness index (NRI) and species richness, mean annual temperature (BIO1), temperature seasonality (BIO4), annual precipitation (BIO12) and precipitation seasonality (BIO15) for angiosperm species along the elevational gradient in Nepal. All variables on the y-axis were transformed to vary from 0 to 1, using the formula $(x - \text{minimum}) / (\text{maximum} - \text{minimum})$. In each panel, filled dots are for NRI. Each dot represents a 100-m elevational zone. The two dash lines in each panel represent the boundaries of the three elevational segments discussed in the main text.



In addition, the Indian plate might also have carried some Gondwanan plant clades that were initially on the Australasian plate and then dispersed to the Indian plate while the latter was moving towards Asia (Raven & Axelrod, 1974). Furthermore, the ‘out of tropical lowlands’ hypothesis (Qian & Ricklefs, 2016) might also account, at least in part, for the observed trend of decreasing phylogenetic relatedness with increasing elevation within the elevational range below *c.* 2000 m a.s.l., a pattern consistent with those observed for angiosperm trees along elevational gradients in tropical regions in Asia and South America (Culmsee & Leuschner, 2013; Qian & Ricklefs, 2016).

Lowland areas in Nepal, particularly those at <1000 m elevation, have been severely transformed by human activity, which would have decreased species richness (Vetaas & Grytnes, 2002). However, the two metrics used in this study are standardized with respect to species richness. The strong and consistent linear relationship between NRI and elevation in the whole elevational gradient below 2000 m in our study, as shown in Figure 1, suggests that human activity at <1000 m in Nepal might have had no or little effect on the pattern of phylogenetic dispersion at <1000 m.

THE MIDDLE ELEVATIONAL SEGMENT (2100–4200 M)

All current elevations between 2100 and 4200 m in Nepal were once lowlands before the India–Asia collision and were uplifted while the Indian plate was under-thrusting the Asian plate. A great body of literature shows that much of the Himalaya–Tibet Plateau was uplifted within the past 15 Myr (Li, 1998), and this region was once occupied by subtropical forests, at least partially, as evidenced by fossil records (e.g. *Cyclobalanopsis* Oerst. in the Upper Miocene; Xu *et al.*, 2016). Because this newly generated elevation segment was once primarily located in subtropical and temperate climates and was occupied by forest vegetation, temperature-based niche conservatism might have played a major role in generating the phylogenetic relatedness pattern (Vetaas *et al.*, 2018), which is consistent with angiosperm phylogenetic relatedness patterns observed in other temperate mountains (e.g. Changbai Mountains in north-eastern China; Qian *et al.*, 2014). The degree of phylogenetic clustering at higher elevations in this elevational segment might have been enhanced by high speciation of certain

clades in the subalpine–alpine ecotone, causing stronger phylogenetic clustering.

THE HIGH ELEVATIONAL SEGMENT (4200–6000 M)

Before the India–Asia collision, the Himalayas had already reached a maximum elevation of 4500–5000 m (Ding *et al.*, 2014; Deng & Ding, 2015) and its low elevations were under a tropical climate (Axelrod *et al.*, 1998). The mechanism described in the ‘out of tropical lowland’ hypothesis (i.e. niche convergence; Qian & Ricklefs, 2016) might have played a dominant role in driving the phylogenetic relatedness of angiosperms, resulting in a trend of decreasing phylogenetic relatedness with increasing elevation. When the initial 5000-m-long elevation gradient was gradually uplifted an additional 4000 m, the mountain-tops were uplifted above the treeline, and forests at high elevations would have given rise to alpine vegetation. After the India–Asia collision, many clades that could not tolerate increasingly lower temperatures would have withdrawn, while species that were able to tolerate both low temperature and strong UV radiation would have evolved from various clades of the original species pool (e.g. *Gentiana* L., *Pedicularis* L., *Primula* L. and *Rhododendron* L.). As a result, the original pattern of decreasing phylogenetic relatedness with increasing elevation would have been retained when the original 0–5000-m elevations were uplifted above the current elevation of *c.* 4000 m, which is approximately the current treeline in the region. The abrupt environmental changes between forest and alpine (non-forest) vegetation (e.g. low sunlight for plants in the forest understorey vs. full sunlight for alpine plants) and the emergence of stronger UV radiation at higher elevations might have driven some distantly related clades that can tolerate high UV radiation (Schön, Martens & van Dijk, 2009) and that possibly favour direct solar radiation (e.g. *Primula* and *Rhododendron*) to evolve many species in the subalpine–alpine ecotone. However, fewer species in each of these clades can tolerate increasingly lower temperatures at higher elevations. As a result, the original pattern of decreasing phylogenetic relatedness with increasing elevation would have been enhanced in the alpine zone.

CONCLUSIONS

Unlike the phylogenetic relatedness of angiosperms along latitudinal gradients, which is driven primarily

Figure 3. Correlation coefficient between the net relatedness index (NRI) and climate variables for 100-m bands in three elevation ranges in Nepal based on climate data obtained from WorldClim (<http://www.worldclim.org>). Climate variables: mean annual temperature (BIO1), temperature seasonality (BIO4), annual precipitation (BIO12) and precipitation seasonality (BIO15).

by temperature-based niche conservatism and increases monotonically with decreasing temperature, that of angiosperms along an elevational gradient might show different patterns among different elevation segments if the elevational gradient is sufficiently long. As we observed in this study, phylogenetic relatedness may decrease, rather than increase, with decreasing temperature above alpine treelines on the one hand and across low elevations on the other, although the same trend in these different elevational segments might be driven by different mechanisms. We propose mechanisms that might have generated the observed zig-zag pattern of phylogenetic dispersion for angiosperms across elevations in Nepal, but future studies should test these when appropriate data become available.

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AUTHOR CONTRIBUTIONS

H.Q. and T.D. conceived the study and performed the analyses, H.Q. and B.S. wrote the paper, O.R.V. contributed ideas, and all authors participated in revising the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. Correlation coefficient between the climate variables (from WorldClim) examined in this study.

Figure S1. Relationship between net relatedness index (NRI) or phylogenetic diversity index (PDI) and elevation for angiosperm genera in Nepal at the genus level, across 100 bootstrap replicates of the phylogeny. Each dot represents the NRI or PDI of all genera in that elevation band, averaged across 100 trees. The error bar indicates the range spanned by 95% of the observed NRI or PDI values from that band.

Figure S2. Relationship between the phylogenetic diversity index (PDI) and elevation for angiosperm species in Nepal.

Figure S3. Correlation coefficient between the net relatedness index (NRI) and climate variables for 100-m bands in three elevation ranges in Nepal based on climate data obtained from CHELSA (<http://chelsa-climate.org>). Climate variables: mean annual temperature (BIO1), temperature seasonality (BIO4), annual precipitation (BIO12) and precipitation seasonality (BIO15).