2016

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Post-print/Accepted manuscript

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Sources of controversy surrounding latitudinal patterns in herbivory and defence

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Abstract. Both herbivory and plant defences against herbivores have been predicted to increase toward tropical regions. Early tests of this latitudinal herbivory-defence hypothesis (LHDH) were supportive, but accumulating evidence has been mixed. We argue that the lack of clarity might be due to heterogeneity in methodology and problems with study design and interpretation. Here we suggest possible solutions. Latitudinal studies need to carefully consider spatial and phylogenetic scale, to link plant defence measurements to herbivore performance, and to incorporate additional concepts from plant defence theory such as tolerance and induced defence. Additionally, we call for consistent measures of herbivory to standardize comparisons across biomes. Improving methodology in future studies of LHDH can resolve much of the current controversy.

Keywords. Biogeography, chemical defence, herbivory rates, macroecology, species interactions.
The Latitudinal Herbivory-Defence Hypothesis

Researchers long have argued that biotic interactions are stronger at equatorial latitudes, so that biotic selective agents drive adaptation in tropical organisms, while abiotic selection pressures more strongly influence temperate organisms [1-3]. This idea is attractive in that it can help explain the remarkable complexity of traits and biological interactions within tropical ecosystems. It could also help explain the striking Latitudinal Diversity Gradient (see Glossary), since coevolution of biotic interactions could drive high diversification rates [3-8] (but see [9]) and prevent competitive exclusion [10]. Many types of biotic interactions can intensify towards the equator [3]; here, we focus on the Latitudinal Herbivory-Defence Hypothesis (LHDH) (Box 1), which summarizes the expected pattern of increasing interaction intensity between plants and their herbivores with decreasing latitude [11, 12] (Box 1). LHDH is one of the most frequently investigated hypotheses about latitudinal gradients in species interactions. Hypothesized mechanisms for LHDH include greater diversity of herbivores [13], more specialist consumers [14], and higher per capita consumption rates at lower latitudes [13]; latitudinal differences in host-plant quality [15, 16]; and reduced abundance of insects at higher latitude [13] due to shorter growing seasons [17], or more top-down control [15]. Yet there is little consensus about whether existing evidence supports LHDH (Box 2).

LHDH began as a comparison of tropical and temperate regions, and was supported by the pioneering work of Coley [11, 18, 19], Levin [20, 21], and their colleagues. However, these authors cautioned that the data should be considered preliminary, given variable sampling techniques and the few existing studies. In the last fifteen years, two trends have emerged as many more studies have been conducted. First, the focus has shifted to include temperate latitudinal gradients in addition to tropical vs. temperate comparisons, such as the landmark
studies of Pennings and colleagues [13, 16, 22-24]. Second, recent reviews and meta-analyses [17, 25], notably by Moles and her collaborators [26-28] have found support for LHDH to be weak or mixed. These findings have stirred controversy, with claims that “ecologists have been slow to re-assess their beliefs” [29] met with counter claims that “statements sounding the death knell” of LHDH are “premature at best” [17].

Here, we argue that much of the disagreement surrounding LHDH (Box 2) can result from heterogeneity among studies in the design chosen and type of data collected. We discuss how even well-designed studies might not be comparable depending on the way herbivory and defence are measured, as well as on their taxonomic and geographic scope. There is therefore a need to carefully consider the implications of different sampling designs, to measure herbivory and defence in appropriate ways (Box 3), and for the plant "defence" traits studied to be justified by a mechanistic link to decreased herbivory (Box 4). We summarize the approaches which have been used to date in original research (Tables 1, S1). Given such heterogeneity in methodology, we think it is still premature to subject the available data to meta-analysis, but we hope that future reviews find enough consistency in study designs to ask how methods affect results. While we focus on latitude, many issues, and many of our recommendations for future research (Box 5), apply generally to studies of geographic variation in biotic interactions.

**Quantifying herbivory and defence**

One of the most common problems identified in reviews of LHDH is the lack of standardized sampling techniques across studies [11, 12, 18, 26, 28, 30]. Although this problem was first mentioned in the literature 25 years ago [11], defence, herbivory, and the fitness consequences of damage are still estimated inconsistently across studies (Table 1, Box 3,4), which can contribute to current contradictions in the field.
Rigorously testing LHDH in a given system requires knowledge of both herbivory and plant defence. Over half of studies on latitudinal patterns in herbivory contained complementary data on defence, nutrients, or palatability, either measured within the paper or in another study of the same system (Table 1). However, the traits evaluated were highly variable between studies (Table S1), which is not surprising since a variety of traits can reduce herbivory [31]. Conversely, researchers might be selecting traits by convenience rather than defensive value. This variation in studied traits makes it challenging to compare across latitudinal studies of defence. Even more problematic, traits which are easily compared across taxa might not be important elements of defence in each species [32].

Inconsistent evidence for latitudinal patterns in defence could be driven by studies of traits that are not actually defensive (Box 4). Many studies measure a variety of plant defence traits without showing that these traits actually deter herbivores. Secondary metabolites assumed to be defence traits can serve other functions in addition to or instead of herbivore deterrence, including allelopathy [33-35], protection from temperature changes [36], UV radiation [37-39], and defence against pathogens [40]. Physical defences such as trichomes [41, 42] can also serve as protection from abiotic stressors (e.g., controlling water loss). Since many environmental characteristics covary with latitude, a latitudinal gradient in leaf traits or chemistry can appear to be a gradient in herbivore defence even when a trait serves a non-defensive function. Therefore, putative resistance traits must be tested for their ability to decrease herbivore performance.

Testing individual traits for their effects on herbivores is difficult because of the great diversity of both potential defences and herbivores. Most plant taxa are not model systems in which the mechanisms and functions of plant defence traits are known, and even if defences are
understood, it is not clear how to compare qualitatively different defences on a standard scale. Greater focus could be placed on secondary metabolites with known anti-herbivore mechanisms (e.g., cardenolides in milkweed) rather than broad classes of chemicals with a range of functions (Box 4). Alternatively, the use of herbivore palatability trials, such as those performed by Pennings and colleagues [16, 23], allows for evaluation and comparison of functional defence without knowledge of underlying traits. This approach can be more informative about defence in non-model systems than haphazard trait measurements [13].

Fitness and tolerance

Although studies of LHDH usually measure tissue consumption, this damage will only have consequences for plant ecology and evolution when it affects plant fitness; that is, fitness provides the vital link between herbivory and the evolution of defence. Damage does not linearly translate to reduced fitness because tolerance can reduce the fitness consequences of herbivory [43, 44]. Additionally, tolerance itself might vary with latitude; for example, if high-latitude plants experience harsher abiotic conditions, the same quantity of damage might have more severe fitness consequences [28, 45]. Accounting for leaf production cost or rate therefore could help to approximate fitness impacts of herbivory and standardize herbivore effects across species [46], but data are needed to show that this approximation is useful. Finally, even higher levels of herbivory should not necessarily lead to stronger evolutionary responses, depending on the relationship between resistance and fitness [12] and the availability of relevant genetic variation. Few studies of LHDH have measured plant fitness (but see [47]), yet incorporating population biology into studies of LHDH is essential for understanding evolutionary consequences of observed gradients in interactions [45].

Spatial and temporal scale of herbivory sampling
Two styles of geographic sampling are often utilized in tests of LHDH: (1) comparisons of high- and low-latitude regions, and (2) comparisons across a latitudinal gradient, involving at least one intermediate site between high- and low-latitude regions (Table 2). For both designs, sampling populations at numerous locations is necessary for a robust test of LHDH. Although some studies have used as few as two locations, most researchers have in fact used much more extensive replication (mean of 25 sites in high vs. low latitude studies and 36 sites in gradient studies: Table 2). As well as the number of sites sampled, replicates must also cover a sufficient geographic range to allow detection of spatial trends.

One might expect that latitudinal gradient studies would cover a smaller latitudinal range because they are more logistically challenging, but of the 34 studies we examined that tested latitudinal patterns in herbivory, the distances covered were found to be nearly equivalent, approximately 16° of latitude on average (Table 2). Nonetheless, absolute distance or latitudinal range is likely less important than the climatic gradient sampled. A shorter transect over a sharp gradient can reveal stronger patterns than a long transect in an environmentally uniform region; for instance, some tropical studies might cover large latitudinal ranges but only small climatic gradients. For these reasons, it is desirable to report information on climate as well as physical transect length. Finally, replicating entire transects in similar biomes in different regions (e.g., temperate forest in Asia, Europe, and the Americas) might also help in describing the generality of any trends. Regardless of approach, increased focus needs to be placed on between site-replication over within site-replication, which in a spatial context is essentially pseudoreplication.

One risk of sampling across a spatial gradient is that uncontrolled environmental cues that trigger phenological events, such as leaf emergence and flowering time, vary with latitude
These cues can lead to unfair comparisons when sites are measured at different stages in their phenology. Tightly controlling for factors that inherently vary with latitude is not possible, but researchers should ensure that sampling does not occur during drastically different parts of plants’ life cycles across sites. Future work should evaluate how the seasonal and developmental timing of herbivory measurements can affect study results [50-52].

The strength or direction of herbivory patterns could also differ within and between years, with rare outbreaks potentially driving selection on defence. Therefore, studies should be replicated between years if feasible. Out of 52 species and communities in the studies that we identified, herbivory was estimated in only one year for 29, in two years for 16, and three to four years for 7 (Table S1). More long-term studies would provide insight into temporal variation in selection on defence, and might explain some of the inconsistencies among short-term studies of LHDH.

Study design

In addition to decisions regarding measurement and replication, selection of study systems can have profound consequences for studying LHDH. Taxonomic sampling (species, communities, or clades), length and location of transects, and the herbivores involved can all influence results.

Single-species studies

Numerous studies have now investigated LHDH for a single species (or several co-occurring species) over a latitudinal gradient [14, 24, 30, 53]; this includes 80% of those examining herbivory rates (Table 1). Such studies are most likely to give straightforward results: since the host plant is constant throughout the gradient, differences in herbivore pressure should be easier to detect, while common gardens and reciprocal transplants can be used to identify
clines in defences [49, 54, 55]. In addition, since the herbivore fauna often is known, patterns of
damage and plant response can be partitioned among particular enemies [30, 56]. Despite these
advantages, single-species studies have limitations. First, many such studies are required to
ensure that results are not species-specific. Second, wide-ranging species are necessary for this
approach, but might not be representative of the majority of species in a community. Third,
transect lengths are limited by species' ranges (e.g., there are no plant species ranging from
lowland tropics to arctic regions). One exception to this last generalization can come from
species occurring in distinctive habitats distributed over long latitudinal gradients. For example,
North American Atlantic salt marshes have provided striking examples of declining herbivory
over very long gradients [13, 16, 22]. The use of congeneric pairs of higher- and lower-latitude
species [23] is one way to broaden the geographic scope of studies while still allowing for
relatively tight phylogenetic control.

Multispecies studies

Multispecies studies address some of the limitations of single-species studies by increasing
taxonomic replication, but they have their own drawbacks. LHDH originally was formulated as a
community-level hypothesis: the prediction of greater herbivory in tropical locations was the
average expectation for the entire community [11, 18]. Some studies have conducted
community-level tests by sampling diverse sets of representative species in two or more
locations [22, 28], while reviews and meta-analyses generally have included any species for
which data are available [11, 17, 18, 25]. Such broad-spectrum approaches are closest to the
original question of whether herbivore pressure is greater in tropical forests, and are essential for
understanding consequences of herbivory for community structure and ecosystem functioning
(e.g., productivity, trophic transfer rates, nutrient cycling). However, care is required in choosing
a representative species set. A sample could be chosen randomly from the local flora [57], but this would be practically difficult in hyperdiverse tropical communities. Another option is to sample the most abundant or ecologically important plants at each study site, regardless of growth form or taxon. This approach recognizes the fact that vegetation type can change along a transect, and is essential for describing community-wide changes in herbivory, but can result in comparisons of taxa as different as trees, grasses, and even lichens [28]. As a result, differences in life history and evolutionary history might obscure patterns evident only in more homogeneous datasets. In addition, the most common species at a site might not be representative of many species in that the community, particularly in the tropics where most species are rare [58].

There are at least three alternative approaches that can reduce the variation associated with sampling very different systems, at the cost of losing information about the average level of damage in a community. First, sampling can be restricted to particular life-forms or functional groups. For instance, by accounting for leaf lifespan, Lim et al. [17] showed evergreen trees followed the expected gradient in herbivory but deciduous trees did not. Second, sampling can be restricted to plants with shared evolutionary histories (e.g., wide-ranging families or genera [51]), although this approach runs into some of the same problems as species-level studies. Third, studies should statistically account for phylogenetic non-independence in the dataset [17, 28, 54, 55]. As an example, the conclusion of Lim et al. [17] that herbivory declined with latitude for evergreens was supported by both non-phylogenetic analyses and (some) phylogenetic regression models. The latter two methods reduce the danger of comparing qualitatively different species because relatives are likely to share defences and life history traits. In addition, phylogenetic control allows for clearer inferences about evolution of defence and diversification.
However, post-hoc phylogenetically controlled analyses might not be effective at controlling for evolutionary history when phylogenies are sparsely sampled and/or replication at the level of genus and family is very low [28]. Additionally, while plant defence is an evolved trait, herbivore damage depends on the entire interacting community; it therefore has been suggested that evolutionary analysis of damage might be inappropriate [17].

Intra- vs. inter-biome comparisons

LHDH originated as a test of differences in herbivory and plant defence between tropical and temperate forests [11, 18, 19]. In contrast, many recent studies of latitudinal trends have been conducted primarily within a single biome, potentially biasing against the detection of climatically-driven patterns. For example, over half of latitudinal herbivory studies have been conducted within the temperate zone, and far fewer studies have taken place in tropical, boreal, or arctic regions (Table 2). Several reviews have collected herbivory rates and defence data from around the globe, but only three studies have collected original data to compare herbivory in tropical and temperate regions (Table S1). Even with global reviews of LHDH, there are usually fewer tropical than temperate sites and species [25, 28].

Tropical systems differ from temperate ones in many ways other than herbivore activity, including host diversity, phenology, leaf lifespan, and nutrient cycling (Box 3). For example, cold-season dormancy at temperate latitudes can result in qualitative as well as quantitative differences in herbivore population dynamics and leaf vulnerability compared to frost-free sites [17, 22]; similar considerations can affect comparisons of highly seasonal tropical habitats with less variable environments. Tropical-temperate comparisons capture the net effect of all such factors, but this raises the issue of whether a transect crossing this biome boundary is comparable to one contained within a single biome. It is possible that no general latitudinal pattern in
herbivory and defence exists within temperate regions [28], but that there still is a clear

difference when tropical and temperate zones are compared [11, 18, 19].

Conducting studies in multiple biomes presents some unique challenges. Taxonomic

overlap between tropical and temperate regions is low, so congeneric and confamilial

comparisons might be needed. In addition, many tropical-temperate transects are interrupted by
different ecosystems (deserts, savannas, mountains). It is perhaps not surprising that latitudinal
trends are difficult to detect if such different systems are included in the same study [28]. Finally,
even within one biome, differences between ecosystems or habitats (e.g., seasonal forest vs.

rainforest; canopy vs. understory) can obscure latitudinal patterns [18]. As a result, latitudinal
trends might be apparent only if comparable habitats are chosen.

Range edge versus latitude

Studies of latitudinal gradients typically confound latitudinal position with distance from a

species' range edge. Herbivory can be reduced at range edges for reasons unrelated to distance

from the equator. Indeed, populations near any range edge might support fewer specialist

herbivores because these enemies are unable to locate or persist in small or isolated marginal

populations. For example, leaf damage by herbivores declined in Asclepias syriaca near both its

southern and northern margins [59]. Crutsinger et al. [60] found that Rhopalomyia rosette galls

of Solidago altissima peaked at mid-latitudes. Finally, populations of the sedge Carex blanda

were less likely to be damaged by fungal pathogens and an insect seed predator near the sedge’s

western range limit [61]. Such effects of range margins could result in a variety of correlations

with latitude if a species were sampled only from its range centre to either latitudinal limit (Fig.

1): the inferred trend could be increasing, decreasing, or none, depending on the latitudinal range

chosen. Instead, LHDH ideally should be tested across a species’ entire latitudinal range.
Differences between responses to range and latitude might also cause problems when comparing single-species and community studies. Single-species studies and multispecies studies should produce the same result only if herbivory declines with distance from the equator both within and among the ranges of the species sampled (Fig. 2a). It is possible that each single-species study could show a decline without any multispecies trend (Fig. 2b) or *vice versa* (Fig. 2c). Likewise, it is possible that the interspecific trend might be a decline with latitude, even if for each species the only effect is reduced damage near its range edges (Fig. 2c).

**Herbivore identity**

A final source of variation among studies can stem from the herbivores themselves. Multispecies studies typically combine impacts of many herbivores to derive an estimate of net damage (Table S1). However, different species occurring on the same plant can show different responses to latitude [22, 30, 56, 62, 63], so that individual species or feeding guilds are not predictive of overall patterns of damage. This means that, if only a subset of a plant's herbivore community is studied, the latitudinal pattern of damage observed might depend on the focal herbivores chosen. As well, the functional composition of the herbivore community itself can vary with latitude. For instance, specialist and generalist herbivores can exhibit different latitudinal patterns [14, 30, 63], and they might respond to plant defences differently. Finally, although most studies have focused on insect herbivores (Table S1), other groups can be important. In particular, vertebrate grazers respond very differently to defences than invertebrates and cause different types of damage [64]. Vertebrates can be more important than insects at high latitudes, suggesting that too narrow a focus on insect herbivores might miss much of the herbivore impact in northern systems.

4. Conclusions and Future Directions
We reject the notion that LHDH is a zombie idea [as suggested by Moles et al. 2016]. Far from being an intellectual quagmire, the debate regarding LHDH represents an ongoing refinement of this field. This debate is a chance to examine how much of this variation between predictions and observed patterns [17, 25, 26, 28] is real versus the potentially informative product of methodological differences (Box 3,4). It is likely that some systems do not follow the expected pattern, but it is premature to judge how often, or under what conditions, plant herbivory and defence are greater at lower latitudes. Better methodology and more careful interpretation of results promise to resolve much of this uncertainty, as well as to provide new insights into underlying issues such as phylogenetic trends, geographic distributions, ecological scaling, and the effectiveness of plant defences.

More ambitious tests with robust metrics for herbivory and plant defence should be carried out at both the community and species levels, across tropical, temperate, and arctic ecosystems [6]; in particular, better sampling of tropical sites is required. Currently, temperate salt marshes are the only habitat that has been thoroughly investigated, and evidence in this system consistently supports LHDH [13, 16, 22-24, 65]. From a macro-evolutionary perspective, large surveys of latitudinal gradients in chemical defences within certain clades have been performed [54, 55], but no comparison has been made simultaneously within and between species of a clade (Fig. 1). While these comparisons represent a logistical challenge, they are necessary to ascertain at what geographic and taxonomic scales LHDH is supported, and how range limits interact with latitudinal patterns.

Resolving the controversy about LHDH has the potential to re-invigorate the study of the mechanisms driving biogeographic variation in herbivory and defence. Tests attempting to determine the mechanisms affecting latitudinal variation in herbivory and defence still are rare.
In addition, the link between herbivory and diversification has been explored in a few clades, but not in the latitudinal context necessary to link LHDH to the latitudinal diversity gradient. We suggest a greater focus on mechanism, and new surveys and experiments with improved methodology, to understand geographic variation in herbivory and defence and how such variation could impact species diversity (Box 5).

**Acknowledgements**

We would like to thank M.T.J. Johnson, the Johnson lab, T. Kursar, D. Schemske, four anonymous reviewers, and P. Craze for helpful comments. Support for this work has come from an NSERC discovery grant to Peter Kotanen, and NSERC Vanier grant to Daniel Anstett.
Box 1 Defining the Latitudinal Herbivory-Defence Hypothesis (LHDH)

The Latitudinal Herbivory-Defence Hypothesis originally postulated that: (1) there is greater herbivore pressure towards the tropics; and (2) in response, tropical plants have evolved higher levels of defences than species at higher latitudes [11, 18, 20]. More intense herbivory could stem from the stably favourable climate of tropical regions which allows insects to feed on plants throughout the year without seasonal reductions in activity or population size, though other explanations have been proposed [1, 11]. For instance, in the tropics, herbivores might also specialize more on particular plant species [66], allowing for more damage even in well defended plants [18].

More recent studies have extended this initial formulation by recognizing that gradients in climate and diversity are globally continuous, so that the arguments of the LHDH extend to latitudinal studies within or between other biomes, rather than just tropical-temperate comparisons [24, 30, 47, 56, 59]. This broadening of perspective has led to much new research, particularly in the temperate zone, but at the cost of diverting attention from the original tropical vs. temperate focus of the LHDH.

LHDH was also initially formulated as community-wide hypothesis, in which herbivory and defence were expected to be greater in tropical forests as a whole, when compared to temperate forests [11, 18]. However, community-level comparisons cannot control for many factors that can influence herbivory apart from latitude (e.g. evolutionary history, growth form); thus, many more recent studies focus on gradients within clades or individual species. Such studies also are valuable because evolution of plant defences occurs at the population level, and will be reflected in similar defence traits within plant genera or family. Here we propose that
clade and community based approaches represent two interrelated but different questions within the LHDH framework.
Box 2. Commonly reported latitudinal patterns

Early work investigating LHDH focused on comparisons between temperate and tropical forests, while more recent research has examined how herbivory and plant defence vary across all latitudes and within a variety of systems. Although some studies support the predictions of LHDH, many studies and reviews of herbivory rates and plant defences have found conflicting patterns [17, 25, 26, 28], raising doubts about the existence of a broad latitudinal pattern predicted by LHDH.

Decline in herbivory with increasing latitude

Many studies have reported decreased damage at higher latitudes [3, 11, 18, 61], a pattern described by LHDH that is often explained by the reduced diversity, abundance, and specialization of herbivores at these latitudes [1, 10, 53]. Herbivore populations at higher latitudes should experience increased density independent mortality, which could lead to decreased damage [45, 67]. Damage might also decrease if the density of hosts declines approaching a plant’s range limit [61, 68-70] (but see [30]).

Greater herbivory at higher latitudes

Contrary to the predictions of LHDH, some studies have found higher herbivory at high latitudes [30, 53, 71-73]. It is often claimed (as LHDH also predicts) that plants at higher latitudes have fewer defences than those in more equatorial locales [1, 2, 11, 74], and that there is less specialization of phytophagous insects in temperate areas [75-77]. Under these circumstances, generalist herbivores in temperate ecosystems might have the potential to consume a large variety and quantity of plant biomass. This effect can be further amplified by herbivore outbreaks, which are believed to occur more often in temperate regions [78, 79], but
see [80]. With less diversity in temperate plant communities, these outbreaks can result in mass defoliations [53].

**No latitudinal pattern with herbivory**

Many studies have found no relationship between herbivory and latitude [28, 30, 57, 81]. As we discuss in this publication, latitudinal patterns can be difficult to detect, but there are also reasons latitudinal patterns truly can be absent. For instance, variation in herbivory can be driven by non-latitudinal differences among species, food webs, environments, or regions, replacing or obscuring any potential latitudinal trends [11, 82, 83].
Box 3. How is herbivory measured?

The ideal approach for a latitudinal herbivory study would be to monitor each leaf over the entire year, and ask whether lower latitude leaves experience more consumption. This approach is hardly feasible, so nearly all latitudinal studies measure a snapshot of standing herbivory on mature leaves instead (Table 1). However, this method typically does not capture complete consumption, and thus has been shown in some studies to underestimate herbivory by about twofold [50, 73]. Therefore, a study of standing herbivory across a latitudinal gradient might falsely reject the LHDH if high rates of complete consumption occur at lower latitudes.

An alternative is to mark and resample leaves to obtain a rate of herbivory over time. This method can capture complete consumption [84], though this must be distinguished from leaf drop [50, 73]. Another advantage is the ability to separately survey mature and young leaf cohorts [81]. Only 12% of latitudinal herbivory studies have measured herbivory on young leaves (Table 1), yet they tend to experience more herbivory than toughened mature leaves [18], and evidence suggests this is especially true for tropical plants [11]. Although some authors argue that standing herbivory on mature leaves should capture young leaf herbivory [53], this assumption does not hold if young leaves are completely consumed. Finally, marking leaves can avoid biased sampling [85], as researchers cannot anticipate future herbivory on selected leaves.

Differences in methods employed in tropical and temperate regions have hampered comparisons of herbivory estimates. Temperate researchers often measure standing herbivory near the end of the deciduous growing season (Table S1), assuming it to equal lifetime damage. While this approach integrates information about the leaf’s entire lifespan, it does not translate well to evergreen species, including many tropical trees, in which leaves can live for years [18] and so might appear to experience more damage in a snapshot measurement simply due to longer
lifespan. Consequently, many tropical researchers estimate herbivory rate using marked leaves [86]. For inter-biome comparisons, it is essential to use comparable methods, ideally including more temperate studies of marked leaves. However, given inter-biome variation in seasonality, leaf lifetime, and leaf production costs, additional methods of standardization might still be necessary to further understand how fitness impacts of herbivory compare between biomes [e.g., 46]. One method that avoids such confounding factors is to estimate herbivore pressure with standard phytometers [22, 87] rather than using naturally-occurring plants.
Many studies of latitudinal variation in defence have relied on imperfect assay techniques or aggregate measures of broad categories of potential defences such as alkaloids or phenolics. This imprecision might obscure latitudinal patterns, or worse, produce spurious trends [49]. Although such imprecise measures can be hard to avoid when comparing broad sets of species, recent advances increasingly are allowing for more informative measurements.

**Individual chemical compounds**

Broad metrics of plant defence compounds, such as total phenolic content, abstract much of the complexity of hundreds of compounds with variable impact on herbivores. Advanced techniques and equipment for chemical analyses now allow for the detailed characterization of these compounds across many samples [88-90]. While the large amount of data can be difficult to interpret, even comparisons of the most abundant compounds can reveal latitudinal patterns that are not evident in total measures. In *Oenothera biennis*, latitudinal patterns in chemical defence which were obscured in total phenolics measurements became clear after the effects of two covarying major compounds were measured separately [49].

**Chemical mechanisms**

In LHDH studies, putative plant defence traits are often measured without testing the defensive function of these traits against herbivores. Establishing mechanisms by which plant defences act can help identify which traits are truly defensive, when multiple traits are correlated with lower herbivory. Such mechanisms have been determined in Solanaceae and Brassicaceae [91], but these families have been underutilized in LHDH studies. Tannins frequently have been considered because of their broad distribution in plants, but often only by simply measuring total concentrations, which confounds compounds involved in defence with those that are not.
Investigation into chemical mechanisms has found that metrics that take into account the pH of insect guts (e.g., total phenolics oxidized) might be more effective [92], and suggest that ellagitannins are better predictors of herbivory than condensed tannins [93].

**Induction and tolerance**

Plant defence induction and tolerance to herbivores have become important concepts in the study of plant defence, yet only three studies of LHDH have directly considered chemical induction [24, 55, 94] and only two studies have considered tolerance [24, 95] across a latitudinal gradient. Trade-offs between constitutive and induced defences, and alternative resource allocation strategies including tolerance, could help explain variation in gradients of plant chemical defence.
Box 5. Key recommendations for experimental design

Much of the current controversy over LHDH stems from the variety of different methods used. Here we provide a list of recommendations for new studies.

1. Herbivory should be estimated in a standardized fashion. One possibility is to standardize by time (Box 2). This can be achieved by monitoring herbivory rates on pre-marked leaves, which also will account for young leaves that are completely consumed by herbivores. Another approach is to use the relative position of sampled leaves to infer leaf age, if the focal plant exhibits a predictable pattern in their emergence. For deciduous plants, studies can focus on the amount of damage leaves incur over their lifetime rather than on the rate [30], although this method complicates comparisons of tropical and temperate herbivory. In this case it might be more informative to compare temperate deciduous to tropical deciduous species.

2. Efforts should be made to measure consequences of herbivory for plant fitness or performance. This might require manipulative experiments in addition to field surveys.

3. Studies should explore the diversity of secondary chemical compounds and quantify individual chemical compounds in addition to broad metrics of plant defence. Measurements of defences at these different resolutions will allow for better understanding of which particular traits are most important in determining trends in overall defence. When possible, studies should focus on defences that have been mechanistically shown to reduce herbivory or should themselves carry out tests for defensive properties. Palatability trials can be an alternative method for quantifying functional differences in defence when important defence traits are unknown. Tolerance and defence induction also should be considered, along with physical traits and life history avoidance strategies [31, 96].
(4) All taxonomic sampling strategies have advantages and disadvantages; therefore, the goals of the study should determine the species set considered. For instance, a study of evolutionary patterns might need to focus on particular clades, while studies relating to maintenance of diversity might require a community approach.

(5) Spatial replication should be sufficient to detect the predicted patterns. When sampling focal plant species or clades, study sites should extend over the entire latitudinal range if possible. It also is desirable to investigate non-latitudinal range edges (longitudinal, altitudinal, etc.), and to compare the effects of population structure or isolation at range limits [45] with latitudinal effects. Climatic or environmental gradients should be reported in addition to transect length.

(6) When possible, studies should identify the key herbivores in the system, allowing clearer interpretation of any trends in damage and defence. Damage rates also should be quantified for different plant tissue types, as these can show contrasting trends [30].
Fig. 1. Two hypothetical patterns of herbivore damage over a latitudinal range; subdivisions i, ii, and iii indicate low latitude, central, and high latitude regions respectively. a) Damage declines linearly with latitude; this might occur when comparing entire communities at different latitudes. Sampling region i, ii, and iii all result in the same trend as sampling the entire range. b) Damage is greatest in the centre of the gradient; this pattern can result for a single species if damage is reduced in marginal populations. Sampling region i, ii, or iii alone result in different trends. In this case, the true pattern can be detected only by sampling the entire range; researchers sampling different regions might correctly describe different, but partial, trends.
Fig. 2. Hypothetical latitudinal gradients in herbivory; each line represents a different species. Damage declines a) with latitude, both within and among species; b) with latitude within but not among species; c) with latitude among but not within species; d) both at each species' latitudinal range margins and with increasing latitude. This figure illustrates how studies over the same latitudinal gradient might produce very different results depending on whether species or communities are considered. Neither result is incorrect, as long as it is correctly interpreted; the discrepancy instead results from the unit of study chosen.
Glossary

**Complete consumption**: Leaves that are entirely consumed by herbivores.

**Latitude**: Absolute distance from the equator. Throughout, we use "low latitude" to refer to more equatorial sites and "high latitude" to refer to more polar sites, regardless of hemisphere.

**Latitudinal diversity gradient**: A biogeographic pattern of biodiversity monotonically increasing toward the tropics.

**Latitudinal Herbivory-Defence Hypothesis**: The hypothesis that there is greater herbivore pressure and that plants are better defended from herbivores toward the tropics.

**Phytometer**: a plant used to assay some component of the physical or biotic environment through its response to experimental conditions.

**Plant defence induction**: The up-regulation of plant defences in response to damage by herbivores.

**Standing herbivory**: Percent leaf area damaged or removed, measured at one particular point in time. Also known as point herbivory.

**Tolerance**: The ability of plants to maintain fitness despite suffering herbivore damage.

**Total phenolics oxidized**: A measure of the activity of plant phenolic compounds that decrease insect fitness by generating oxidative stress (formation of free radicals and other reactive compounds). This occurs when they are oxidized under the alkaline conditions characteristic of insect digestive systems.
Table 1. Counts of qualitative characteristics of original latitudinal comparisons of herbivory rates, excluding reviews and analyses of existing data. A total of 34 such studies were identified. Studies and communities were counted twice if both categories applied, and not counted if methods were unclear. See also Online Supplementary Material Table S1 for a list of studies and details.

<table>
<thead>
<tr>
<th>Total</th>
<th>Taxonomic sample</th>
<th>Growth form</th>
<th>Life history</th>
<th>Deciduousness</th>
<th>Herbivory method</th>
<th>Leaf age</th>
<th>100% consumption</th>
<th>Leaf traits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Comm.</td>
<td>Woody</td>
<td>Herb</td>
<td>Peren.</td>
<td>Annual</td>
<td>Decid.</td>
<td>Ever.</td>
</tr>
<tr>
<td>By species</td>
<td>52</td>
<td>45</td>
<td>7</td>
<td>35</td>
<td>17</td>
<td>48</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>By study</td>
<td>34</td>
<td>27</td>
<td>7</td>
<td>24</td>
<td>12</td>
<td>33</td>
<td>3</td>
<td>22</td>
</tr>
</tbody>
</table>

Key: Comm. = community, Peren. = perennial, Decid. = deciduous, Ever. = evergreen, Yes = measured, No = not measured

Table 2. Geographic coverage of 34 original latitudinal studies of herbivory rates, excluding reviews and analyses of existing data. See also Online Supplementary Material Table S1 for a list of studies and details.

<table>
<thead>
<tr>
<th>No. studies</th>
<th>Lat. span (°)</th>
<th>No. sites</th>
<th>No. sites/degree lat.</th>
<th>Studies per biome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>High vs. low lat.</td>
<td>10</td>
<td>17</td>
<td>6-44</td>
<td>25</td>
</tr>
<tr>
<td>Gradients</td>
<td>24</td>
<td>15</td>
<td>4-79</td>
<td>36</td>
</tr>
</tbody>
</table>

Key: Lat. = latitude, Trop. = tropical (<23°N/S), Temp. = temperate (23-55°N/S), Boreal = 55-66°N, Tundra > 66°N
References


27. Moles, A.T. and Ollerton, J. (2016) Is the notion that species interactions are stronger and more specialized in the tropics a zombie idea? Biotropica 48, 141-145


34. Fraenkel, G.S. (1959) The raison d'etre of secondary plant substances; these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. Science 129, 1466-1470


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<th>Page</th>
<th>Reference</th>
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