

LETTER

Can genetically based clines in plant defence explain greater herbivory at higher latitudes?

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Abstract

Greater plant defence is predicted to evolve at lower latitudes in response to increased herbivore pressure. However, recent studies question the generality of this pattern. In this study, we tested for genetically based latitudinal clines in resistance to herbivores and underlying defence traits of *Oenothera biennis*. We grew plants from 137 populations from across the entire native range of *O. biennis*. Populations from lower latitudes showed greater resistance to multiple specialist and generalist herbivores. These patterns were associated with an increase in total phenolics at lower latitudes. A significant proportion of the phenolics were driven by the concentrations of two major ellagitannins, which exhibited opposing latitudinal clines. Our analyses suggest that these findings are unlikely to be explained by local adaptation of herbivore populations or genetic variation in phenology. Our results show that greater herbivory at high latitudes can be explained by latitudinal clines in the evolution of plant defences.

Keywords

Biogeography, climatic gradient, common garden, ellagitannins, flowering time, herbivory, latitude, macroecology, *Oenothera biennis*.

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INTRODUCTION

One of the most common and striking biogeographic patterns of terrestrial environments is a change in biotic interactions towards lower latitudes. Many species interactions have been predicted to increase in intensity due to warmer temperatures, longer growing seasons and a greater diversity and biomass of herbivores (Dobzhansky 1950; Coley & Aide 1991; Coley & Barone 1996; Schemske *et al.* 2009). These stronger species interactions are expected to lead to greater selection and evolution in traits that mediate species interactions. Plant–herbivore interactions have long served as a model for testing these predictions, where it is expected that herbivory and selection on plant defence increase towards lower latitudes. This hypothesis is called the Latitudinal Herbivory Defence Hypothesis (hereafter LHDH) (Coley & Aide 1991; Johnson & Rasmann 2011).

Empirical studies investigating latitudinal gradients in herbivory and defence have found mixed results in support of the predicted pattern. Early studies comparing tropical to temperate forests found evidence for increased herbivory and defence in the tropics (Levin 1976; Coley & Aide 1991; Coley & Barone 1996). This early work is valuable for providing the first empirical results on the topic, but early studies often lacked standardised sampling and adequate replication across a range of latitudes (Johnson & Rasmann 2011; Moles *et al.* 2011a). Some recent studies do show the predicted increase in

herbivory and defence at lower latitudes (Hallam & Read 2006; Pennings *et al.* 2009; Pearse & Hipp 2012; Lehndal & Agren 2015), while other studies show no latitudinal pattern (Kelly *et al.* 2008; Sinclair & Hughes 2008; Adams *et al.* 2009; Woods *et al.* 2012), or even higher herbivory and defence at higher latitudes (Adams & Zhang 2009; Steinbauer 2010; Moles *et al.* 2011b; Hiura & Nakamura 2013). These conflicting results have led to controversy in the field about potential biases associated with studies that find support for or against the hypothesis (Moles *et al.* 2011a; Moles 2013).

Additionally, there are several assumptions of LHDH that are problematic. One important assumption is that putative resistance traits causally affect the preference or performance of herbivores. A recent meta-analysis showed the surprising result that many chemical ‘defence’ traits do not consistently predict resistance to herbivores in the field, whereas traits not typically thought of as defences (e.g. flowering time) frequently have a strong effect on resistance to herbivores (Carmona *et al.* 2011). Thus, it is necessary to measure many types of resistance traits in a single study and to explicitly assess their role in defence. A second assumption is that plants in regions with higher herbivory should have higher defence. This assumption is problematic on two counts: (1) plants that are more highly defended because of greater investment in resistance traits often exhibit lower damage, not higher; and (2) plants may evolve alternative defensive strategies that do not influence the amount of damage, such as

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tolerance (Strauss *et al.* 2002). Additionally, LHDH assumes that all herbivore species will cause more damage at lower latitudes, which is unlikely. Individual herbivore species are likely to vary in their abundance and corresponding damage to plants across latitudes due to many species-specific factors, such as life history, feeding guild, adaptation to climate, the amount of standing genetic variation within insect populations and population dynamics. Any of these aspects of insect biology may cause herbivory on a single plant species to be greater at higher latitudes (e.g. Hiura & Nakamura 2013). In testing the LHDH, it is important to explicitly test the assumptions described above.

The present study extends on our recent research into biogeographic patterns of herbivory on common evening primrose (*Oenothera biennis* L., Onagraceae). We previously surveyed 79 populations of *O. biennis* across the entire north-south native range, which encompassed 17 degrees of latitude (Anstett *et al.* 2014). Latitudinal patterns in herbivory varied according to the feeding guild and degree of specialisation of the herbivore species. When assessing leaf herbivory, stem boring damage and seed predation, all possible linear relationships were found (i.e. positive, negative and no pattern) between herbivore damage and latitude. Although our study identified environmental predictors of these patterns, we were unable to determine whether these patterns were caused by phenotypically plastic or genetically based clines in either the traits of the herbivore species or the plant *O. biennis*.

Building on our previous work, we test for genetically based latitudinal clines in plant resistance to three herbivore guilds (leaf chewers, xylem feeders and floral/fruit herbivores). We collected seeds from 137 populations of

O. biennis across the entire range of the species (Fig. 1), and grew replicate plants from each population in a single common garden environment. We measured 26 chemical, physical, life history and physiological traits, including natural herbivore damage and insect abundance, to answer the following research questions: (1) Is there a genetically based latitudinal gradient in resistance to herbivores? (2) What plant traits best predict latitudinal patterns in resistance against a diversity of herbivores? And, (3) what environmental variables best predict observed latitudinal patterns in herbivory and resistance traits? Overall, this study provides insight into why herbivory can increase with latitude. To provide further phylogeographic context to our results we examine the haplotype diversity and structure of populations sampled across the range of *O. biennis*.

MATERIALS AND METHODS

Experimental site and study system

We conducted the study at the Koffler Scientific Reserve (KSR). This 350-ha field station is located in the Oak Ridges Moraine, 50 km north of Toronto, Canada (www.ksr.utoronto.ca). The focal plant *O. biennis* is common at the site.

Oenothera biennis is a herbaceous forb native to eastern North America where it grows along roadsides, old fields and at the margins of lakes, rivers and oceans (Dietrich *et al.* 1997). This plant is functionally asexual due to a genetic system called permanent translocation heterozygosity (Dietrich *et al.* 1997). A diverse and well-characterised community of generalist and specialist herbivorous insects attack *O. biennis*

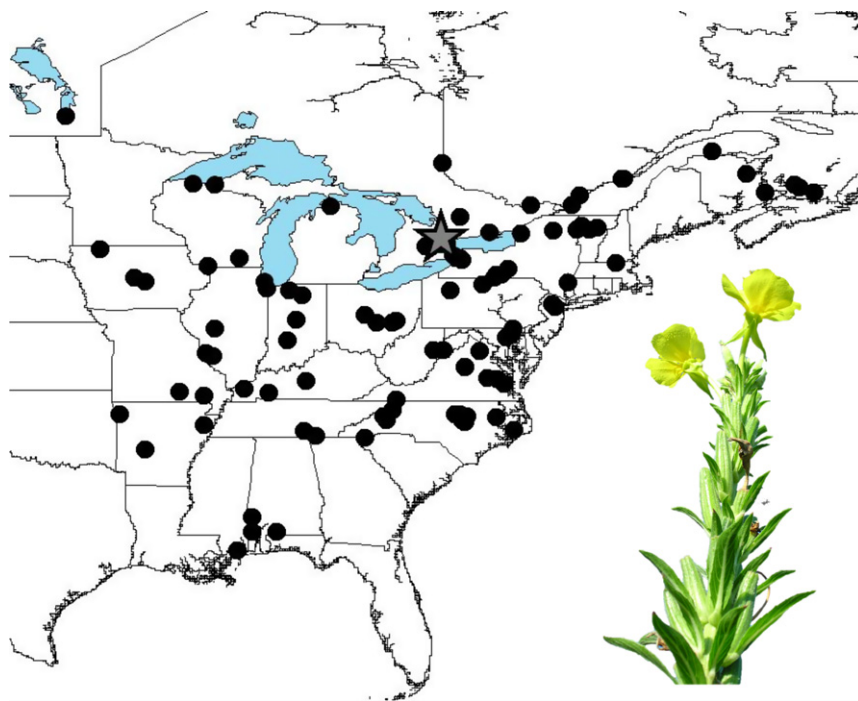


Figure 1 The native range of *Oenothera biennis* and localities of populations. Each point represents the locality of one of the 137 genotypes that were included in the study. The black and grey stars represent the location of the common garden. A single *O. biennis* plant is shown in the lower right.

(Johnson & Agrawal 2005), making this system a good model for the study of plant–insect interactions.

We focused our study on leaf herbivory, the abundance of a xylem feeder and fruit damage caused by common specialist herbivores of *O. biennis*. Leaf herbivory is caused by a diversity of generalist and specialist herbivore species (Johnson & Agrawal 2005). We quantified abundance of the xylem-feeding meadow spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), which is the most common early season generalist herbivore. Late in the season we quantified damage by the specialist evening primrose moth *Schinia florida* G. (Lepidoptera: Noctuidae), which feeds on developing flowers and fruits. We also quantified damage by the specialist micromoth *Mompha brevivitella* C. (Lepidoptera: Momphidae), whose larvae consume developing seeds before pupating in the fruit and emerging as an adult in the fall. *Schinia florida* and *M. brevivitella* have direct impacts on fitness through their consumption of seeds, and recent research shows that these herbivores drive rapid evolution of flowering time and fruit chemical defences within local populations (Agrawal *et al.* 2012). These specialists are also found throughout the range of *O. biennis* (Anstett *et al.* 2014).

Phenolic chemistry

Oenothera biennis is a model for the study of phenolic metabolism. Apart from a diversity of flavonoids, *O. biennis* produces a range of ellagitannins, some of which are unique within the plant kingdom (unpublished data). Ellagitannins are a group of hydrolysable tannins that are reactive within the alkaline conditions of insect guts, where they are thought to cause oxidative stress (Barbehenn *et al.* 2006; Salminen & Karonen 2011). Ellagitannins may also be oxidised before entering the gut by plant polyphenol oxidases and peroxidases, or they may bind and precipitate dietary proteins in the non-alkaline pregut region (Salminen 2014). Past research has shown that there is genetic variation in the concentrations of most ellagitannins both within and between species (Johnson *et al.* 2009; Johnson *et al.* 2014). Moreover, multiple phenolic metabolites predict resistance against herbivores and experience ongoing natural selection (Johnson *et al.* 2009; Agrawal *et al.* 2012). How phenolics vary within *O. biennis* at larger geographic scales to affect herbivores is not understood.

Common garden

Seeds of genotypes from 137 populations were collected from across the native range of *O. biennis* (Fig. 1) and germinated on filter paper. Five replicate plants were then grown in 25 mL biodegradable pots for 2–3 weeks. The plants were watered daily as needed and 0.25 g of slow release fertiliser pellets (Nutricote Total 13-13-13; Chiiso-Asahi Fertilizer Co., Tokyo, Japan) were added to the soil surface of each pot. On 5 August 2012, the pots were planted directly into the ground of an old field at the Koffler Scientific Reserve that was mowed once before planting. Plants were arranged into rows and columns with one meter spacing, and the rows and columns were divided into five adjacent spatial blocks to allow for the partitioning of environmental variation. After

planting, plants were left to grow and overwinter. In April 2013, any genotypes that had bolted the previous year were replanted using the aforementioned procedure. A fence was built around the common garden which effectively reduced deer herbivory.

In this study, seeds were utilised from field and laboratory collections, which could be problematic if maternal effects are prominent. However, plants were germinated simultaneously and grown in a common environment for 3 weeks before transplanting into the field, and measurements were not taken until the second year of growth. These methods have been shown to decrease maternal and epigenetic effects in many plant species (Galloway 2001; Agrawal 2002) including *O. biennis* (Gross & Kromer 1986). Importantly, maternal effects that affect herbivores are typically short-lived, having their largest effects on young plants (Agrawal 2002). Since we assessed plant defence well into the second year of growth, the contribution of maternal effects are expected to be minor. Epigenetic modifications have not been directly studied in *O. biennis*, but they are likely to be small in contrast to the large amount of genetically based heritable variation in phenotypic traits (Johnson *et al.* 2009; Agrawal *et al.* 2012; Hollister *et al.* 2015). Past research on the population genetics of *O. biennis* shows that most populations contain a single plant genotype, whereas geographically separated populations typically contain distinct plant genotypes (Levin 1975; Larson *et al.* 2008; Hollister *et al.* 2015).

Trait measurement

We measured 22 plant traits including life history, physical, physiological and chemical traits that have been implicated in resistance against herbivores. The date of first flowering was monitored throughout the growing season. Growth rate was measured by taking the difference between the first height measurement > 5 cm and the next height measurement 5–12 days later, divided by the number of days in between measurements. Final height was measured at the end of the season in early October. Leaf toughness was quantified as the force required to puncture a fully expanded leaf using a force gauge penetrometer (Type 516; Chatillon, Kew Gardens, New York, USA). The number of trichomes present on the upper surface of a 5 mm diameter disc taken from a fully expanded leaf was counted using a dissecting microscope. Specific leaf area was measured from each plant by dividing the surface area of a 5-mm disc by its dry mass. Percent water content of fully expanded leaves was measured as: $(\text{Mass}_{\text{wet}} - \text{Mass}_{\text{dry}}) / \text{Mass}_{\text{wet}} + 100\%$. Drying occurred by placing the leaves in a drying oven at 50 °C for 48 h.

Leaf, flower and fruit tissues were collected for chemical analysis of total phenolics, oxidative capacity of phenolics and the concentrations of two hydrolysable tannins (oenothein A and B) involved in chemical resistance. The detailed methods for these chemical analyses are provided in Appendix S1.

Herbivory

Resistance to herbivores was measured as the amount of damage to leaf and fruit tissues or total number of individuals

in the case of *P. spumarius*. Leaf herbivory was visually estimated in early September as the percentage area consumed on 10 leaves evenly distributed across the plant. *Schinia florida* damage was measured as the number of fruits damaged divided by the total number of fruits. *Mompha brevititella* damage was measured as the number of adult exit holes present on 10 randomly selected fruits, where each exit hole corresponds to a single larva.

Phylogeography of *O. biennis*

To provide a phylogeographic context to our study, we examined the distribution and diversity of haplotypes sampled from across the native range of *O. biennis*. Specifically, we characterised haplotype diversity (richness, Simpson's index) across 70 populations of *O. biennis* by sequencing the hypervariable trnL-rpl32 intragenic cpDNA region from 119 individuals collected from 70 populations across the range of *O. biennis* (Fig. S2). Our detailed methodology and results are presented in Appendix S2.

Weather data

Temperature and precipitation data were downloaded from the Worldclim database (Hijmans *et al.* 2005). Values for annual temperature (bio1), temperature range (bio7), annual precipitation (bio12), precipitation seasonality (bio15) and altitude were downloaded at a resolution of 10 km². All layers were projected using the Behrmann equal-area projection. Values from each layer were extracted for the GPS locations of each of the 137 populations using ArcGIS. Distance to common garden was estimated to test whether insect herbivores were locally adapted to populations closest to the common garden. This was measured as the Euclidean distance between each population and the common garden.

Statistical analysis

A combination of bivariate and multiple regressions were conducted to examine patterns in herbivory and plant traits using R (R Core Development Team 2012) (see Appendix S3). All traits were regressed individually against latitude using linear regressions as implemented by the *lm* function in R. We also regressed herbivory traits against distance to common garden while accounting for genetic variation in plant traits. We used default settings. We then used linear regression to test which resistance traits predicted herbivore damage. Within major classes of comparisons (e.g. herbivory vs. latitude), sequential Bonferroni corrections were made for multiple comparisons.

We used both ordinary least-squares and generalised linear models to perform multiple regressions. Environmental variables were used to predict herbivory and plant defence. Plant resistance traits and distance to common garden were also used to predict herbivory. Competing models were selected using the *dredge* function in the *MuMIn* library in R (R Core Development Team 2012). Δ AIC was computed for each model by subtracting the AIC of a given model from the lowest AIC. Models with Δ AIC < 2 were then used in the dredge model averaging procedure. All data were checked for homo-

geneity of variance, normality and influential data points using alternative data transformations (i.e. log (x + 1), square root and squared) before the final analysis. Points that were more than 4 SD from the predicted value were considered outliers and removed from analyses. Variables that did not meet statistical assumptions were log (x + 1) or square-root transformed as appropriate; transformations are shown in Table S1. Negative-binomial generalised linear models were used when assumptions of linear models were not satisfied. All variables used in *lm* models were standardised to a mean of 0 and standard deviation of 1.

RESULTS

Genetically based clines in herbivory and plant traits

We detected consistent genetically based clines in resistance to generalist and specialist herbivores. The amount of herbivory a plant genotype received was positively related to the latitude of origin of the genotype for all herbivores examined (i.e. genotypes from higher latitudes had more herbivory; Fig. 2, Table S1). Leaf herbivory by generalist and specialist herbivores (slope = 0.07, P < 0.001, R^2 = 0.09), and the average number of *P. spumarius* (slope = 0.10, P < 0.001, R^2 = 0.17) were greater on genotypes from higher latitudes. Similarly, damage by the specialist *S. florida* (slope = 0.07, P = 0.001, R^2 = 0.08) and *M. brevititella* (slope = 0.22, P < 0.001, R^2 = 0.34) were higher on genotypes from higher latitudes.

Plant phenolics frequently showed genetically based latitudinal clines. The concentration of total phenolics in fruits increased towards lower latitudes (Fig. 3a, slope = -0.13, P < 0.001, R^2 = 0.23), while leaf and flower total phenolics showed no significant pattern with latitude (Table S1). Fruit oxidative capacity also increased at lower latitudes (slope = -0.11, P < 0.001, R^2 = 0.18), while leaf oxidative capacity increased at higher latitudes (slope = 0.08, P < 0.001, R^2 = 0.12) and flower oxidative capacity showed no latitudinal pattern (slope = 0.02, P = 0.44, R^2 = 0). Concentrations of the two dominant phenolic compounds (oenothein A and B), showed opposing latitudinal patterns (Fig. 4, Table S1), where the trimer oenothein A was higher at lower latitudes across all tissues (Fig. 4a, c, e), and the biosynthetically related dimer oenothein B was greatest at higher latitudes across all tissues (Fig. 4b, d, f). These opposing patterns were in part explained by a genetic trade-off in the production of oenothein A and B for genotypes collected at different latitudes (Fig. S1). The oxidised form of oenothein A showed similar albeit weaker patterns to non-oxidised oenothein A (Table S1).

Genotypic variation in flowering phenology, physical and physiological traits associated with leaves was also related to latitude. Flowering date (Fig. 2b, slope = -0.16, P < 0.001, R^2 = 0.43) and specific leaf area (SLA) (Fig. 2c, slope = -0.06, P = 0.006, R^2 = 0.06) were both negatively correlated with latitude, such that plants from more southern localities flowered later and had leaves that were less dense (i.e. lower SLA). Trichome density increased at lower latitudes (slope = -0.2, P = 0.022, R^2 = 0.04), but this pattern is not significant after Bonferroni correction. No other trait was

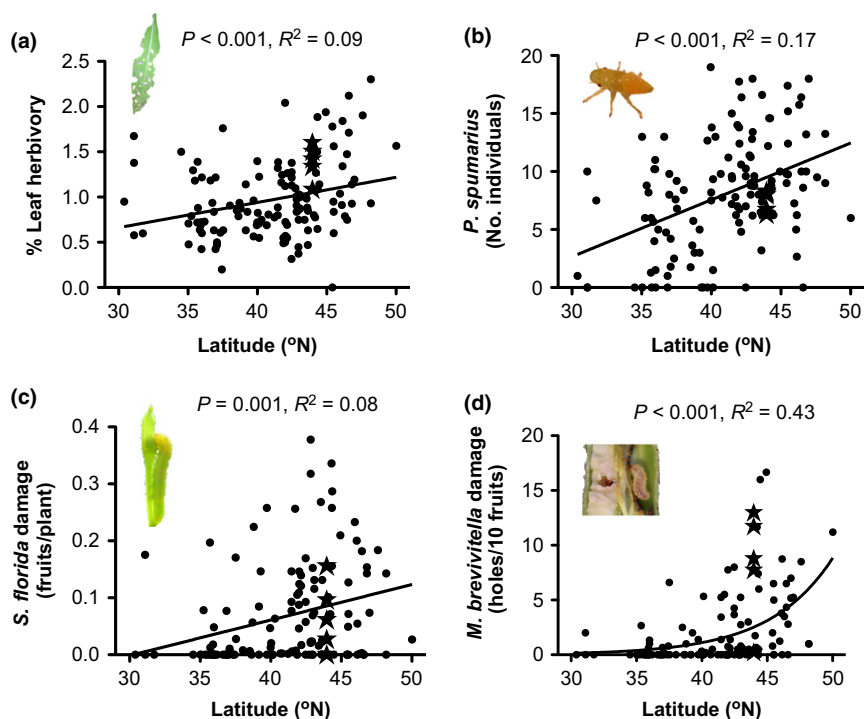


Figure 2 Genetically based latitudinal clines in resistance to herbivores on *Oenothera biennis*. Each point represents the mean of one distinct genotype, where herbivory measurements were averaged across five replicate plants. Regressions are shown for (a) generalist leaf herbivory, (b) *Philaenus spumarius* abundance, (c) % fruits damaged by *Schinia florida* and (d) the number of fruits impacted by *Mompha brevitella* per 10 fruits. (a–c) are linear regressions, while (d) utilises a negative-binomial generalised linear model (GLM). Stars indicate populations that are closest to the common garden.

related to latitude (Table S1). Multiple regression of environmental variables revealed that mean annual temperature was the main predictor of genotypic variation in herbivore damage and variation in plant traits (Table S2–S5). Temperature range and precipitation variables were rarely significant.

Plant traits that predict resistance to herbivores

Multiple plant traits predicted resistance against herbivores in multiple regression analyses (Table S6–S7). Flower total phenolics (slope = -0.63 , $P = 0.004$, $R^2 = 0.08$) was negatively related with *S. florida* damage, while flowering date (slope = -0.04 , $P < 0.001$) and flower total phenolics (slope = -3.4 , $P = 0.003$) were negatively related to *M. brevitella* damage. *Philaenus spumarius* abundance also showed a negative relationship with flowering date (slope Fig. S1 = -0.23 , $P = 0.039$, $R^2 = 0.12$), flower total phenolics (slope = -0.43 , $P = 0.016$, $R^2 = 0.04$) and SLA (slope = -0.42 , $P < 0.001$, $R^2 = 0.16$). Leaf herbivory was only related to final height (slope = -0.33 , $P = 0.001$, $R^2 = 0.14$). Oenothien A and B showed particularly strong correlations with all herbivory types (Fig. 5), but they were not included in multiple regressions due to strong correlations with total phenolics (Appendix S4). Additional traits were related to herbivory from the different species, but they showed weaker relationships or clearly represented spurious relationships (e.g. wrong tissue for that type of herbivore) (Table S7). Furthermore, pairwise correlations showed that many additional traits not detected

in multiple regressions showed relationships with herbivory (Fig. 6, Table S8). In addition, distance to common garden did not affect resistance to any herbivore types while accounting for variation in plant resistance traits in a multiple regression (Table S10–S11). This result suggests that herbivores present at the field site are not locally adapted to the closest populations.

Genetic diversity across latitudes

A total of 14 distinct cpDNA haplotypes were found across the entire native range of *O. biennis*. We found evidence of two distinct lineages separated by seven mutational steps (Fig. S2). The more diverse southern lineage largely occupies areas south of the regions covered by the Wisconsin glacier, whereas relatively few haplotypes are abundant in previously glaciated regions (Fig. S3, Appendix S3).

DISCUSSION

Our study provides clear results relating to the evolution of plant resistance across a latitudinal gradient, which may help to resolve conflicting patterns of herbivory and defence reported from other studies. We detected opposing genetically based clines in herbivory and resistance traits in a common garden field study. All herbivores inflicted more damage on genotypes taken from higher latitudes and this pattern was explained by clines in chemical resistance, flowering phenology

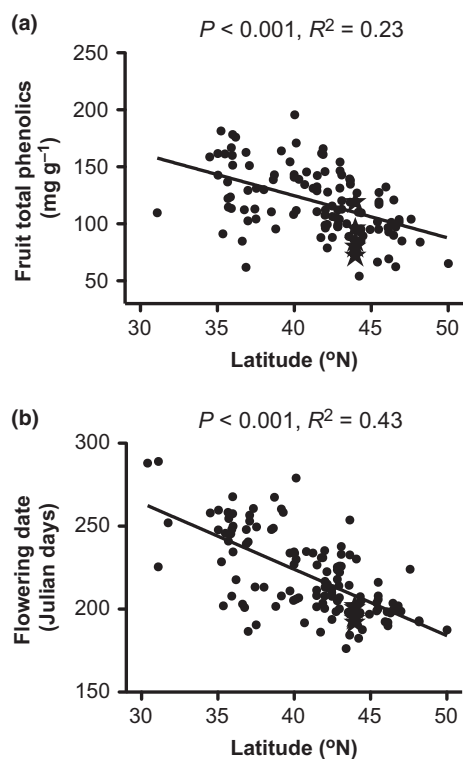


Figure 3 Genetically based latitudinal clines in resistance traits of *Oenothera biennis*. Each point represents one distinct genotype, where herbivory was averaged across five replicate plants. Stars indicate populations that are closest to the common garden.

and leaf physiological traits, where genotypes from southern latitudes were better defended. These results support the prediction that plants evolve greater defence at lower latitudes, and this can in part explain why *O. biennis* populations experience greater herbivory by some herbivore species at high latitudes (Anstett *et al.* 2014).

Latitudinal gradients in herbivory

The simplicity of the LHDH has been useful to bring about the investigation of latitudinal clines in plant–herbivore interactions at a global scale. Yet, many studies report greater herbivory at higher latitudes, which is opposite to the predicted pattern (Kelly *et al.* 2008; Kozlov 2008; Adams & Zhang 2009; Hiura & Nakamura 2013; Anstett *et al.* 2014; Kim 2014). For example, in a previous study we showed that natural populations of *O. biennis* exhibited higher herbivory at higher latitudes in three specialist flower and fruit feeding herbivores (Anstett *et al.* 2014). The results presented in the present study suggest that herbivory may be greater at higher latitudes because plant resistance is lowest in these regions. We showed that total phenolics and specifically oenothain A are higher at lower latitudes. Moreover, these traits predict lower herbivory in a common garden that controls for environmental variation (Table 2, Fig. 2). If this association is causal and common in other systems, it would suggest that some of the early predictions of the latitudinal–herbivory

defence hypothesis should be revisited (Coley & Aide 1991; Johnson & Rasmann 2011; Moles *et al.* 2011a). Indeed, it logically follows that the evolution of higher resistance with decreasing latitude should lead to the opposite pattern in herbivory.

Research that integrates patterns of herbivory and underlying resistance traits are important to understand biogeographic patterns of plant–insect interactions. Various studies consider only latitudinal patterns in herbivory with no measure of plant traits (Kelly *et al.* 2008; Crutsinger *et al.* 2013; Kim 2014). Other studies have only measured putative resistance traits, without testing which traits influence herbivores (Levin 1976; Stark *et al.* 2008; Ardón *et al.* 2009; Pearse & Hipp 2012; Moreira *et al.* 2014). A limited number of studies have considered both herbivory and resistance in the same system, but they have not yielded a consistent pattern (Siska *et al.* 2002; Adams *et al.* 2009; Pennings *et al.* 2009; Woods *et al.* 2012; Hiura & Nakamura 2013). For example, studies of salt marshes showed higher herbivory and defences at lower latitudes (Siska *et al.* 2002; Pennings *et al.* 2009; Więski & Pennings 2014). By contrast, higher herbivory was found at the range centre of *Asclepias syriaca*, while putative resistance traits showed no latitudinal pattern or increased with latitude (2012). Hiura & Nakamura (2013) found mixed latitudinal patterns in herbivory on *Fagus crenata* (Japanese beech), while putative defences were greatest at higher latitudes (2013). To the best of our knowledge, our study is the first to show that lower defences at higher latitudes explain opposing patterns of herbivory. An important next step will be to test whether other observations of higher herbivory at higher latitudes (Kelly *et al.* 2008; Kim 2014) are explained by opposing latitudinal clines in resistance traits.

Latitudinal gradients in chemical resistance

Previous studies examining latitudinal gradients in chemical resistance found inconsistent latitudinal patterns. Many studies support the prediction of greater chemical defences at lower latitudes (Levin 1976; Coley & Aide 1991; Siska *et al.* 2002; Hallam & Read 2006; Rasmann & Agrawal 2011; Pearse & Hipp 2012), while others found no pattern or higher defence at higher latitudes (Moles & Westoby 2003; Adams *et al.* 2009; Ardón *et al.* 2009; Martz *et al.* 2009; Steinbauer 2010; Moles *et al.* 2011b; Hiura & Nakamura 2013). A meta-analysis found that only 16% of studies showed greater chemical defences at lower latitudes (Moles *et al.* 2011a). A limitation of previous studies is that most assume a role of anti-herbivore resistance in their traits without actually testing how these traits functionally affect herbivore preference or performance.

Our study provides several explanations for the lack of consistency in latitudinal patterns of plant defence. We observe that while key traits that predict herbivory show increased levels at lower latitudes (e.g. oenothain A), other traits seem to correlate positively with herbivory and latitude (e.g. oenothain B). Our results for latitudinal patterns in resistance traits only support LHDH in the context of traits that explain decreased levels of herbivory. Otherwise, if herbivory measurements had been omitted from this study, we would have con-

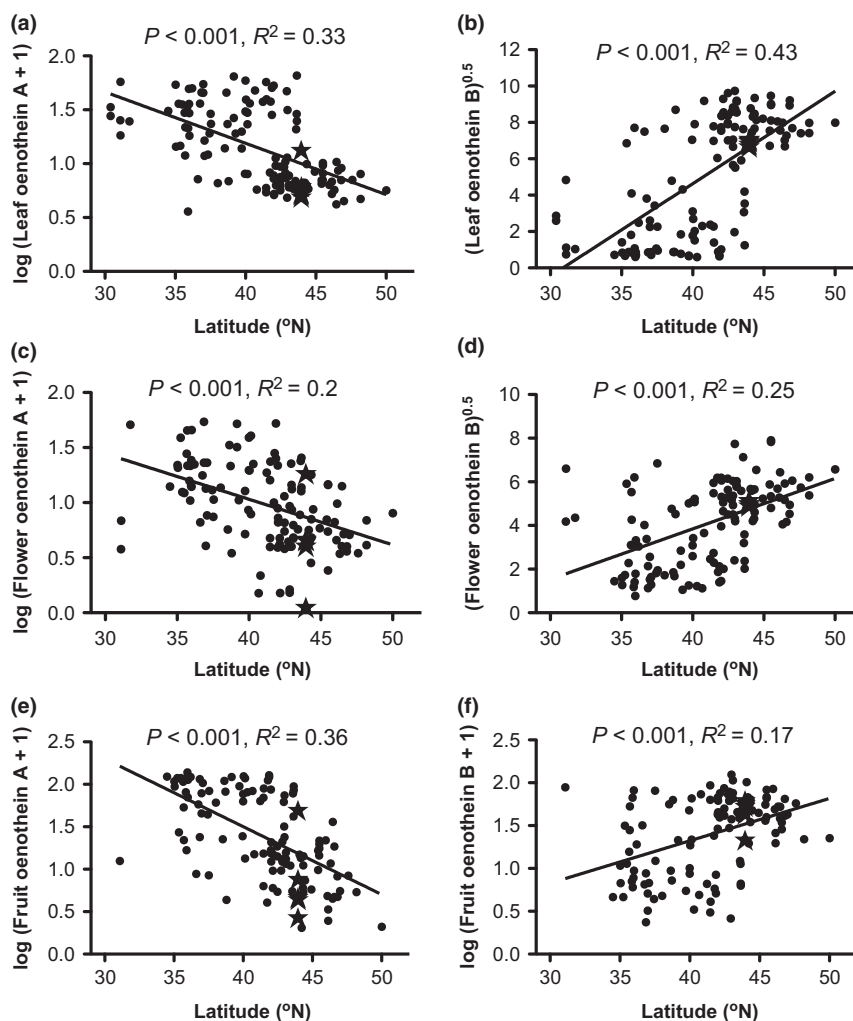


Figure 4 Latitudinal patterns in the two major compounds found in *Oenothera biennis*. Oenotherin A and B concentrations are shown across three different tissues. Each point represents the genotypic mean across five replicate plants. Stars indicate populations that are closest to the common garden.

cluded that plant defence traits show no consistent latitudinal pattern. This emphasises the value of measuring both herbivory and a wide diversity of resistance traits within the same study, since many putative plant resistance traits may not actually decrease herbivory.

Oenotherin A and B are the predominant phenolics in tissues of *O. biennis*. These specific metabolites are particularly relevant to the defence of *O. biennis* against herbivores (Johnson *et al.* 2009; Agrawal *et al.* 2012). The trade-off between these two compounds likely added noise to the data, which may have prevented total phenolics from showing a stronger pattern, particularly in leaf and flower tissues. Indeed, stronger latitudinal patterns and stronger prediction of herbivory occur across more tissues when oenotherin A is used rather than total phenolics or oxidative capacity (Table S1). This shows how detailed chemistry measurements can provide a more accurate test of the hypotheses and greater insight into the underlying mechanisms driving plant–herbivore interactions.

Recent methods have been developed to allow for the measurement of oxidative activity of tannins, which is thought to more closely represent the functional mechanism by which

tannins reduce herbivore performance by creating oxidative stress in insect guts (Appel 1993; Salminen & Karonen 2011). Interestingly, total phenolics revealed latitudinal patterns only in fruits, while the oxidative capacity revealed latitudinal patterns in two tissue types (leaves and fruits). Our results do not explicitly allow us to determine whether *O. biennis* herbivore defence is mediated via oxidative reactions or protein precipitation. However, since phenolics lose their ability to effectively bind proteins at high pH (like that found in insect guts) (Appel 1993), and ellagitannins are abundant in *Oenothera* and well known for their oxidative activity (Salminen *et al.* 2011), it is most likely that at least part of the defensive function of these chemicals occurs through oxidative stress and may help to explain some of the genetically based clines in resistance.

Many traits other than plant chemistry are known to have roles in deterring herbivory (Carmona *et al.* 2011). We found that flowering time was an important predictor of herbivory, which is consistent with previous findings that show flowering phenology is among the most important defences in this system because early flowering genotypes avoid attack by

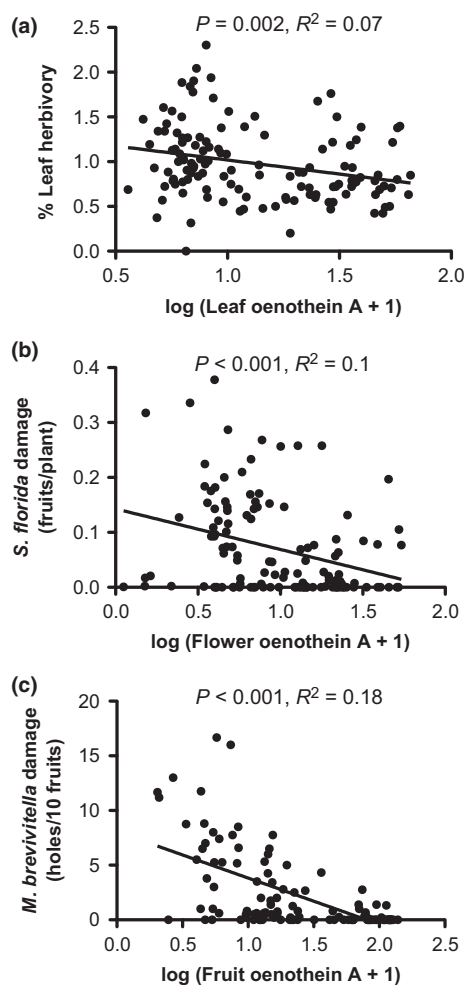


Figure 5 Genotypic correlation between oenothain A and herbivory across three different tissues. Each point represents the genotypic mean across five replicate plants.

specialist flower and fruit herbivores (Agrawal *et al.* 2012, 2013). SLA also emerges as a predictor of *P. spumarius* abundance which likely reflects the fact that this organism depends on properties of leaf tissues and xylem conductivity. However, chemical resistance appears to be a more important predictor, since it exhibits larger effect sizes and is more often significant in multiple regression comparisons of all resistance traits (Table S6). Most other traits do not predict resistance, consistent with previous findings (Johnson *et al.* 2009).

Phylogeography

The distribution of haplotype diversity found in this study (see Appendix S2) supports the idea of significant genetic differentiation across a latitudinal gradient. Our results support previous predictions of higher genetic diversity in more southern regions (Levin 1975) (see Appendix S3). Haplotype diversity is higher in previously unglaciated regions (Fig. S2) and lower in previously glaciated areas. Given the genetically based cline in defence, these patterns suggest that low resistance genotypes colonised previously glaciated regions, or

lower levels of defence evolved following Northward migration of highly dispersive haplotypes (Appendix S2).

Limitations of the study

While we have presented a rigorous test of LHDH, some limitations of our study exist. Since only one garden was used, it is possible that phenological variables, photoperiod or climate would differentially affect plant resistance traits at the latitude of origin of individual genotypes. However, this is unlikely to change our main results and conclusions since many resistance traits did predict herbivory in similar ways to those reported from previous work on *O. biennis* (Johnson *et al.* 2009; Agrawal *et al.* 2012), and were congruent with patterns seen in fruit tissues during a latitudinal survey of natural populations (Anstett *et al.* 2014). Moreover, multiple regression showed that the effect of flowering time is either not a significant predictor of resistance or has a much smaller effect size when compared to other plant resistance traits (Table S6). Thus, any mismatches in plant and insect phenology are unlikely to explain our results.

A second limitation is that our use of a single common garden makes it difficult to differentiate the roles of genetically based clines in plant resistance vs. local adaptation of insect populations. To address this issue we performed analyses that tested whether the distance of a plant genotype from the common garden predicts resistance to herbivores, which would be the expected pattern if insects were adapted to local genotypes. We find no significant relationships once variation in plant resistance traits is taken into account (Table S10–S11). This suggests that genetically based latitudinal clines in plant defence best explain latitudinal variation in resistance to herbivores.

CONCLUSIONS

We have shown that herbivory on *O. biennis* exhibits a genetically based cline with greater susceptibility at higher latitudes and greater resistance at lower latitudes. This pattern in herbivory is explained by clines in chemical and non-chemical resistance traits, whereby plant genotypes at southern latitudes are better defended than genotypes from northern latitudes. Our findings may help to resolve past conflicting results by showing that latitudinal patterns in resistance traits and herbivory may frequently show opposing patterns. These results and our previous work (Anstett *et al.* 2014) illustrate the importance of studying patterns of herbivory in natural populations in combination with dissecting the genetic basis of specific defensive mechanisms against herbivores.

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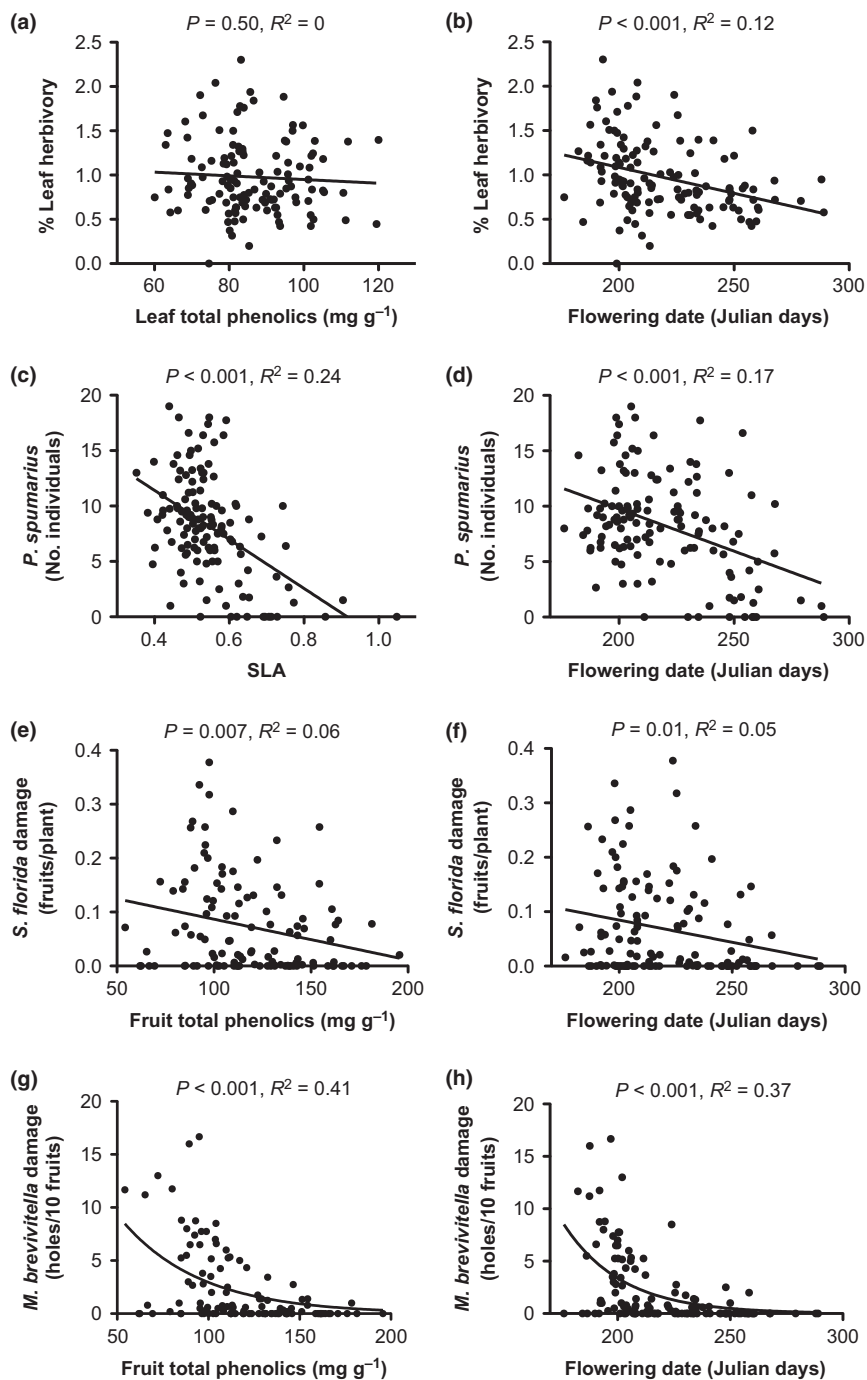


Figure 6 Plant resistance traits that predict herbivory on *Oenothera biennis* across four herbivory types. Results of linear regressions are shown for all herbivory types except *Mompha brevitella*, where a negative-binomial generalized linear model was used. Each point represents the genotypic mean across five replicate plants.

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AUTHORS CONTRIBUTIONS

DA contributed to the ideas, design, data collection, analyses and interpretation. JA and JPS contributed to the ideas, novel methods, data collection and interpretation. JG con-

tributed to data collection and analysis. NN analysed the phylogeographic data. MJ contributed seeds and helped to develop the ideas, experimental design, data analysis and interpretation. All authors contributed to the writing and editing of the manuscript.

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