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Transgenerational effects alter plant defence and resistance in nature

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Abstract

Trichomes, or leaf hairs, are epidermal extensions that take a variety of forms and perform many functions in plants, including herbivore defence. In this study, I document genetically determined variation, within-generation plasticity, and a direct role of trichomes in herbivore defence for *Mimulus guttatus*. After establishing the relationship between trichomes and herbivory, I test for transgenerational effects of wounding on trichome density and herbivore resistance. Patterns of interannual variation in herbivore density and the high cost of plant defence makes plant–herbivore interactions a system in which transgenerational phenotypic plasticity (TPP) is apt to evolve. Here, I demonstrate that parental damage alters offspring trichome density and herbivore resistance in nature. Moreover, this response varies between populations. This is among the first studies to demonstrate that TPP contributes to variation in nature, and also suggests that selection can modify TPP in response to local conditions.

Introduction

By exposing plants to a variety of environments, Clausen, Keck and Heisey developed the common garden experimental design to distinguish between genetically determined, environmentally dictated, and genetic × environmental effects on growth and fitness (Clausen *et al.*, 1948). This ability for an individual to react to an environmental stimulus and alter its growth, development or state is termed ‘phenotypic plasticity’ (Bradshaw, 1965; West-Eberhard, 1989; Via *et al.*, 1995). Phenotypic plasticity comes in many forms, often classified as being either active or passive, adaptive or nonadaptive, and continuous or discrete (West-Eberhard, 2003). When an environmental cue is a reliable predictor of future ecological conditions, and a plastic response increases fitness in that condition, the given plasticity is advantageous and expected to evolve (West-Eberhard, 1989; Agrawal, 2001a; Herman *et al.*, 2014; Kuijper & Hoyle, 2015). In this way, the current environment acts as a source of information from which an individual can use to modify future development (English *et al.*, 2015).

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Following the hypotheses regarding within-generation plasticity, if current environmental conditions are a good predictor of the conditions experienced by the next generation (positive intergenerational autocorrelation), then the transmission of altered developmental trajectories between generations (transgenerational phenotypic plasticity, TPP, Fig. 1c,d) should also be adaptive (Herman & Sultan, 2011; Herman *et al.*, 2014; Jablonka, 2013; Leimar & McNamara, 2015). For example, if annual herbivore patterns exhibit positive autocorrelations across two generations, selection should favour genotypes of plants that, when exposed herbivory, produce offspring transgenerationally primed for herbivore defence (Hoyle & Ezard, 2012; Prizak *et al.*, 2014). However, if the variability does not exhibit any autocorrelation over the intergenerational timescale, TPP is expected to evolve to a zero or slightly negative level (Kuijper & Hoyle, 2015). With this conceptual framework, this manuscript addresses TPP to wounding in *Mimulus guttatus* and considers the role of parental environment and local adaptation on offspring trichome density and herbivore resistance in the field.

Since the demonstration of transgenerationally induced defences in wild radish (*Raphanus raphanistrum*) (Agrawal *et al.*, 1999), plant–herbivore interactions have become a model system for studying TPP. Agrawal *et al.* (1999) demonstrated that the progeny of

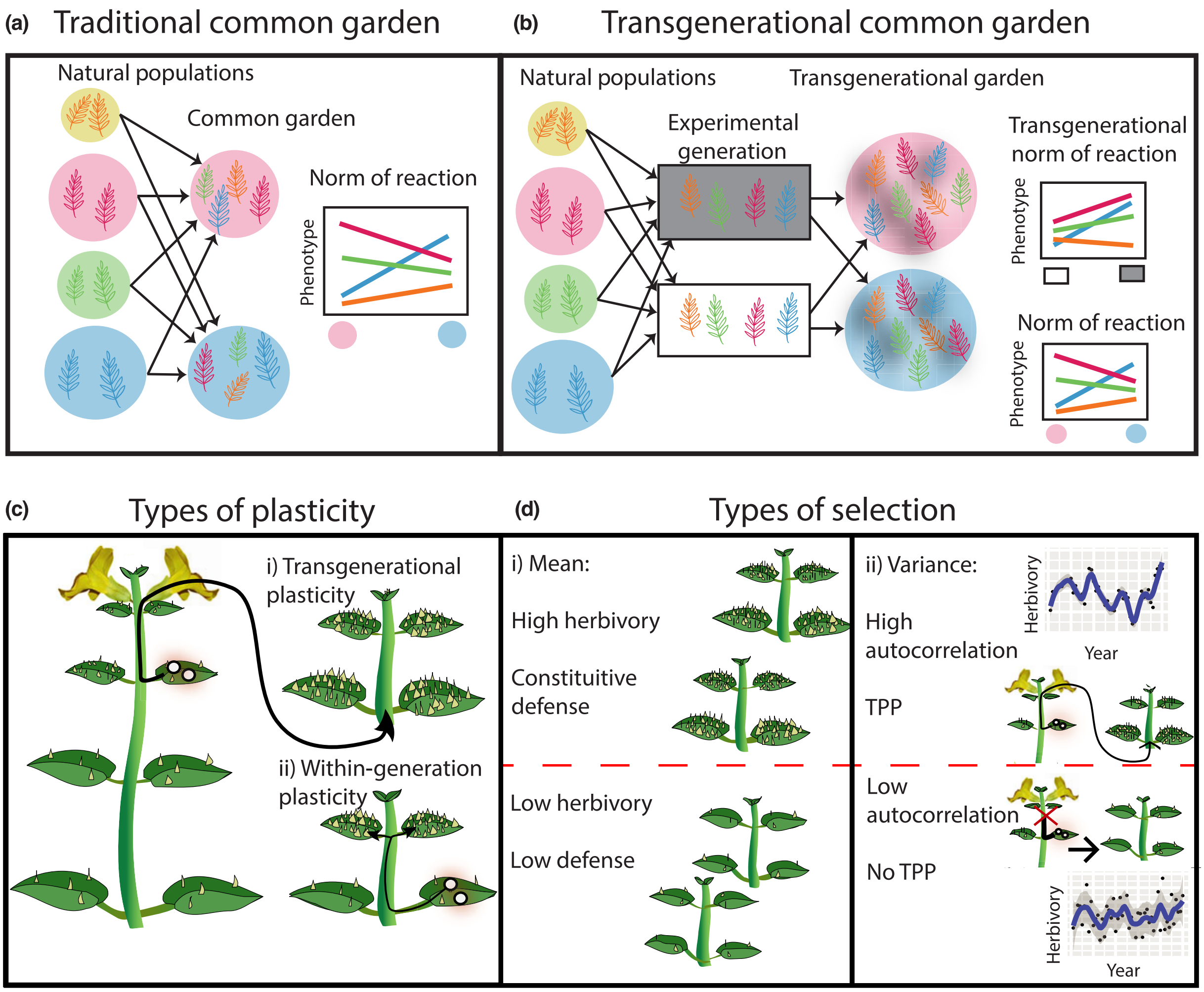

Fig. 1 Diagram of a traditional common garden experiment (a) compared with the transgenerationally extended common garden experiment (b) utilized in this study. Both experimental designs allow genetic, environmental and genetic × environmental effects to be parsed out through norms of reaction and associated analyses. The extended transgenerational approach utilizing an experimental generation (b) allows for transgenerational effects to be considered within this framework. This framework allows transgenerational plasticity (c.i) to be considered alongside traditional within-generation plasticity (c.ii) to study damage-induced trichome production in *Mimulus guttatus*. While constitutive trichome densities are expected to evolve in accordance with mean herbivore abundance at a given site (d.i), it is the presence or absence of interannual autocorrelations in herbivore abundance that are expected to select for or against transgenerational plasticity of trichome induction (d.ii).

plants exposed to caterpillar herbivory were themselves more resistant to caterpillars (and that *Daphnia* exposed to predators produced better defended offspring), accelerating the study of TPP on biotic interactions (Agrawal *et al.*, 1999). Numerous studies across taxa have since demonstrated that the offspring of wounded plants produce more chemical and physical defences than the offspring of control plants (Agrawal, 2001b, 2002; Holeski, 2007; Holeski *et al.*, 2012; Rasmann *et al.*, 2012; Ballhorn *et al.*, 2016). This framework is conceptually tied to the optimal defence theory (McKey, 1974), which predicts that the costs/benefits and probability of attack provide information to the plant which selects for plants to defend various organs at levels that maximize fitness under local conditions. In this context, parental wounding provides an additional source of information that a plant can utilize to produce appropriate levels of defences given the cost/benefit trade-off. However, the optimal defence theory also predicts a trade-off in constitutive vs. induced defences (McKey, 1974; Fritz & Simms, 1992; Zangerl & Rutledge, 2015), which is not necessarily predicted through this information theory framework. Instead, the mean levels of herbivory over time would be expected to select for baseline constitutive levels of defence, whereas the patterns of variation in herbivore frequencies would select for or against within-generation plasticity or TPP. In other words, constitutive and inducible defences, respectively, reflect the elevation and slope of the norm of reaction of defence on variation in herbivory.

Concurrent with theoretical advances of when TPP should be advantageous (Herman *et al.*, 2014; Kuijper & Hoyle, 2015), recent studies of the transcriptional basis of TPP (Colicchio *et al.*, 2015b), its epigenetic origin (Boyko *et al.*, 2010; Lang-Mladek *et al.*, 2010; Verhoeven *et al.*, 2010; Calarco *et al.*, 2012; Rasmann *et al.*, 2012; Herrera & Bazaga, 2013) and taxonomic prevalence (Holeski *et al.*, 2012) have confirmed the complex and widespread role of TPP in plant defence. In *M. guttatus* (yellow monkeyflower), simulated herbivory (mechanical leaf wounding) to parental plants leads to offspring with more trichomes – defensive hair-like epidermal structures – than offspring of undamaged parental plants (Holeski, 2007). The offspring of wounded plants differ from the offspring of control plants via a broad, multifaceted transcriptional response (Colicchio *et al.*, 2015b). Molecular epigeneticists have recently demonstrated the presence of an intricate three-part system through which histone modifications (Greenberg *et al.*, 2013), DNA methylation (Matzke & Mosher, 2014) and small RNAs (Sunkar *et al.*, 2007; McCue *et al.*, 2012) are responsive to environmental conditions (Dowen *et al.*, 2012), alter gene expression (Eichten *et al.*, 2014) and persist into the following generations (Verhoeven & van Gurp, 2012). These epigenetic mechanisms appear likely to represent the underlying basis of transgenerational plasticity (Rasmann *et al.*, 2012), although maternal nutrient provisioning and seed coat modifications have also been implicated in TPP (Galloway, 2001; Luzuriaga *et al.*, 2006). While this paper focuses on TPP's role in plant–herbivore interactions, the role of TPP in evolution extends to many environmental variables (Bossdorf *et al.*, 2008), and across a wide diversity of life, including a significant breadth of the animal kingdom (Lancaster *et al.*, 2007; Donelson *et al.*, 2012; Gapp *et al.*, 2014; Woestmann & Saastamoinen, 2016).

Although the molecular underpinnings, and the theoretical ramifications of TPP, have become increasingly understood, field studies addressing the role of transgenerational effects in nature are still relatively sparse. In *Campanulastrum americanum,* maternal light conditions impact the life history strategy (annual vs. biennial) of their offspring in a way that increases the fitness of the next generation if it too is grown in the same light condition (Galloway & Etterson, 2007, 2009). In *Phaseolus lunatus* (wild lima bean), a recent study utilized a similar common garden approach to the one applied here to study transgenerational chemical defence induction and the role of this induction on plant survival in nature (Ballhorn *et al.*, 2016). However, interpopulation variation in TPP was not considered in either of these studies. Work outside of the plant–herbivore defence literature has demonstrated that genetic differences exist that alter the role of parent environment on offspring phenotype (Schmitt *et al.*, 1992; Sultan, 1996; Vu *et al.*, 2015), but these studies have not tested the effects of transgenerational effects in field conditions or in regard to insect herbivory.

Previous studies in the *M. guttatus* system have demonstrated the presence of transgenerational trichome induction to wounding, and variation in this response across recombinant inbred lines (RILs) and their progenitor inbred lines (Holeski, 2007; Holeski *et al.*, 2010; Scoville *et al.*, 2011; Colicchio *et al.*, 2015b). *Mimulus guttatus* is fed on by a wide range of Lepidoptera, including both generalists and specialists (M. Rotter and L. Holeski, in review), and produces a diverse suite of phenylpropanoid glycosides (PPGs) (Keefover-Ring *et al.*, 2014) that can provide additional levels of herbivore defence or attraction (Holeski *et al.*, 2013). While the parental stress imposed in those experiments and the one used here, mechanical damage through hole-punching, does not perfectly mimic herbivory (Reymond *et al.*, 2000), at least in *Arabidospsis*, the transcriptomic responses to mechanical damage and herbivore damage do overlap significantly (Reymond *et al.*, 2000).

Over the past decade, the genetic (Holeski, 2007; Holeski *et al.*, 2010) and transcriptional (Scoville *et al.*, 2011; Colicchio *et al.*, 2015b) basis of TPP in *M. guttatus* has become better understood, but until this point no one has tested for a role of trichomes or TPP in herbivore defence within the *M. guttatus* system. Here, I utilize an extended common garden approach (Fig. 1b) to gain insight into the function of *M. guttatus* trichomes, the role of TPP on plant–herbivore interactions in nature, and population-level differences in transgenerational plasticity.

Methods

Natural population phenotyping, herbivory assays and collection

During the summer of 2014, I identified 16 natural populations of annual *M. guttatus* within a 150 by 50 mile area in Central Oregon. These sites ranged in elevation from 89 to 1481 m (Table S1). When over 50% of plants at a given site began setting seed (between 7 June and 5 August), 12–20 plants were collected per site and brought to the Plant Biology laboratory at HJ Andrews Experimental Forest. Here, I assayed herbivory on every leaf of the primary axis on a 0 to 5 ranking (0: no leaf damage, 1: 1–10% leaf area removed, 2: 11–25%, 3: > 26%) using a visual estimation of leaf damage that has been found accurate (Johnson *et al.*, 2015). I also measured plant height and width and counted the number of flowers produced by each plant. In addition, I counted trichomes from three leaves per plant (one of which was always of the second leaf pair, and two of which were later leaves) as described previously (Colicchio *et al.*, 2015b). At the end of the growing season (10 August–2 September), I revisited these sites and randomly selected and collected seed from 10 plants per site.

Experimental glasshouse generation

In the fall of 2014, I grew seed from six maternal lines for eight of the 16 populations sampled in the field (chosen to represent a wide range of ecological conditions) at the University of Kansas glasshouse. Seeds were germinated individually in one-inch cells, before being transplanted to two-inch containers for continued growth. I phenotyped one-third of the plants for second leaf trichome density at the third leaf pair expanded stage. Of the remaining plants, half were subject to damage through the hole-punch method from the third through the sixth leaf pair, which was previously shown to induce increases in trichome production in inbred *M. guttatus* lines (Holeski, 2007; Colicchio *et al.*, 2015b). Within each population, I randomly selected one damaged and one control plant from each maternal line to use as parent plants for the next generation, whereas the remainders were phenotyped for 2nd and 7th leaf pair trichomes. Plants derived from the location ‘Trailbridge Road’ (TBR) did not continue leaf development to the 7th leaf pair and were therefore excluded from the analysis of within-generation trichome induction. I performed crosses using a circular crossing design for both damaged and control breeding individuals. Briefly, a wounded plant from maternal line one was used as the pollen donor for a cross with a wounded plant from maternal line two. This same wounded plant from maternal line two was then used as a pollen donor for a cross with a wounded plant from maternal line three, and so forth, until a wounded plant from maternal line six was used as the pollen donor for the original wounded plant from maternal line one. This same exact pattern was repeated with control plants, and done for each of the eight populations. From this, I generated six paired lines for each of the eight populations, deriving from either the offspring of two control or two damaged plants (96 lines total).

Field common garden design

During the summer of 2015, I germinated seeds from each of these populations at the University of Oregon glasshouse in 1″ flats and transplanted 1- to 2-week-old seedlings into two common garden sites in the Cascade mountain region of Central Oregon. At both sites, individuals were planted in a randomized design across the site. One common garden, HJ Andrews Experimental Forest (HJA), was located > 2 mi away from any native *M. guttatus* population and differed from typical *M. guttatus* habitat in that it contained a greater composition of soil organic matter and received fewer hours per day of direct sunlight. The other site, Browder Ridge (BR), features a large native population and has been the site of numerous prior common garden experiments (Mojica *et al.*, 2012; Monnahan & Kelly, 2015).

At HJA, 1232 seeds from 44 lines (four were excluded due to insufficient seed set), each replicated with either damaged or control parents, were planted on 18 April, 799 (64.8%) germinated and were planted into the field on 12 May, with 224 of these plants (28.0%) eventually flowering. A total of 1056 seeds from these same lines were planted for the BR garden on 4 May, 573 (54.3%) germinated and were transplanted into this field site on 25 May, and of these, 94 reached flowering (16.4%). During the growing season, rapid dry-down due to the drought conditions in the Cascades during the summer of 2015 necessitated the addition of supplemental water at both sites at the rate of 5 gallons per common garden site every other day for 3 weeks during the growing season. Of the surviving 318 plants, 153 were the offspring of damaged parent plants, whereas 165 were the offspring of control plants. Trichome counts were completed for 271 of 318 plants.

I surveyed plants every other day, and on the day that a plant produced its first flower, the following traits were scored: largest leaf length and width, number of leaves, plant height, node of flower, peduncle length. I assayed herbivory by the same method as described above, and one second node leaf was collected for trichome phenotyping.

Glasshouse common gardens

To test whether the observed transgenerational effects persisted in glasshouse as well as field conditions, the same offspring of damaged and control plants were grown at the University of Kansas glasshouse during the fall of 2015 and spring of 2016. Seeds were germinated and grown in one-inch cells, and when plants reached the third leaf pair expanded stage, a second node leaf was collected and phenotyped for trichome density as described above.

Statistical analysis

Natural field survey

Population-level analysis. Incidence of herbivory (percentage of leaves at a site with any herbivore damage), elevation, aridity (Zomer *et al.*, 2008), latitude and longitude were all considered as possible explanatory variables in a least-squares regression of population mean second leaf pair trichome density in JMP v10 (SAS Institute Inc., Cary, NC, USA). Significance in this model was determined through *t*-tests. The linear relationship between population incidence of herbivory and trichome density was carried forward using linear regression. The same approach was used with incidence of herbivory as the response variable, and elevation, aridity, latitude and longitude as explanatory variables. Correlations between both population mean field trichome density, and glasshouse trichome density, with population incidence of herbivory were calculated in JMP.

Leaf-level analysis. Mixed models were constructed using the (R:lmee:glmer) package followed by ANOVAS comparing models to test whether trichome densities affected severity or likelihood of damage in this model (Bates *et al.*, 2014). Within this model, population and individual leaf trichome densities were treated as fixed effects, with plant nested within population treated as a random effect. The response variable was either a binary variable of whether or not the plant received and wounding (binomial family model), or an ordinal variable representing the severity of wounding (Poisson family model). All leaves (654) were considered for the binary herbivory or no herbivory model, whereas only leaves with at least minor herbivory (320) were considered to test for an effect of trichomes on limiting herbivore severity.

Trichome/fitness relationship. Least-squares regression was used to determine the relationship between population, trichome density (averaged across the three leaves per plant), a 'population × trichome' interaction term and stem width on plant flower production. Stem width was included in this model as a covariate to help partition out variation due to general plant vigour, and get more directly at the relationship between trichome density and flower production for a plant of a given size. Effect significance was determined by *F*-ratio tests based on factor sum of squares.

Within-generation plasticity

For analyses with trichome density as the response variable, I transformed the densities as log (trichome density + 1). Trichome counts were right-skewed with a large number of 0 values. To detect signatures of phenotypic plasticity, log (7th leaf trichome density + 1) was treated as the response variable with population, family nested within population, damage treatment and a 'population × damage' interaction term explanatory variables. Using a GLM framework in JMP v10 (SAS Institute Inc.), log-ratio chi-square tests were performed between models to determine significance. The residuals in this model were approximately normally distributed and were not correlated with predicted trichome density. Subsequently, the data were split according to population and GLMs were fit with the above terms to test whether individual populations did in fact exhibit significant within-generation plasticity.

Transgenerational plasticity

Population of origin, line nested within population, growth environment (field or glasshouse), parental damage, and all possible two interaction effects between population, parental damage and growth environment, as well as their three-way interaction were considered in a GLM model with log (2nd leaf trichome density + 1) as the response variable. Using JMP v10 (SAS Institute Inc.) log-ratio chi-square tests were performed between models to determine the significance of the various terms. The residuals in this model were approximately normally distributed and were not correlated with predicted trichome density (Fig. S1). Subsequently, the data were split according to population and GLMs were fit with the above terms to test whether specific populations exhibit significant transgenerational plasticity.

Considering all leaves, I created a model to test which, if any, factors limit the likelihood that a plant experiences herbivore damage. I used a stepwise model selection method in JMP v10 (SAS Institute Inc.), with herbivore wounding coded as binary response variable, and possible explanatory variables to include were population, site, parental treatment, days to flower, leaf width, plant height, trichomes, treatment × population, site × treatment, site × trichomes and site × population as possible explanatory factors. Considering leaves from which there was at least some sign of insect damage, I created a model to test which, if any, factors limit insect herbivore damage, using a stepwise model selection method in JMP v10 (SAS Institute Inc.), with severity of damage coded as minor, moderate or high as the response variable, and the same possible explanatory variables. For both models, I then selected a model using both a minimum BiC and AiCc criterion.

Environmental autocorrelations in the Oregon Cascades

Mean annual temperature and precipitation data from the northernmost, southernmost, westernmost and easternmost populations within this study system between 1895 and 2014 were downloaded at 4 sq. km. resolution using the data explorer tool from the PRISM climate group (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, 12 February 2016). Autocorrelations were calculated using R:acf (R Core Team, 2014) at a lag of one.

Results

Natural population surveys

Central Oregon *M. guttatus* exhibit vast natural variation in trichome density (7.5 trichomes/cross section: 127.5 trichomes/cross section, Table S1) and experience quite different magnitudes of leaf herbivory (8% of leaves experiencing herbivory: 67% of leaves,

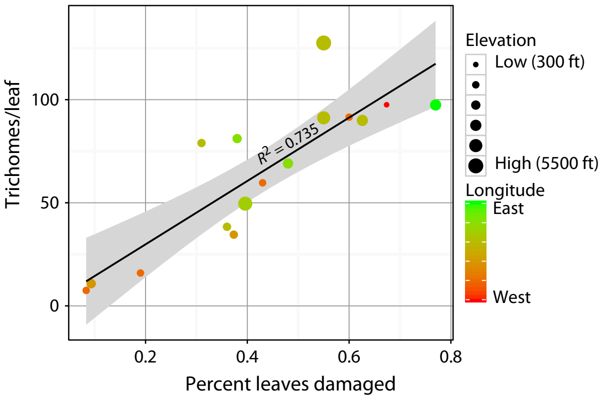

Fig. 2 Scatterplot and regression of population mean trichome density to the percent leaves damaged at a given site. Point size is coded to represent the elevation of the site and colour to the longitude, both of which were not found to be significantly correlated with leaf trichome density.

Table S1). Incidence of herbivory at a site was the only factor that significantly covaried with population mean trichome density in a least-squares regression ($t_1 = 3.96$, $P = 0.0027$, Table S2) when considered alongside latitude, longitude, aridity and elevation. Additionally, incidence of herbivory was not significantly correlated with any of the preceding environmental variables (Table S3). The strong positive correlation between population incidence of herbivory and population mean trichome density at native field sites ($R^2 = 0.735$, Fig. 2), as well as population mean trichome densities measured in the glasshouse ($R^2 = 0.82$), suggests that herbivory-driven natural selection plays a role in driving population trichome density variation.

Although plants in the two different common garden locations did significantly differ in the probability that a leaf would receive herbivore damage (mixed-model logistic regression: $\chi^2_{15} = 100.85$, $P < 9 \times 10^{-15}$, Fig. 3a), trichomes did not alter the likelihood that a leaf would receive herbivore damage ($\chi^2_1 = 0.23$, $P = 0.63$). On the other hand, trichomes did significantly reduce the severity of herbivory on leaves that showed at least minimal signs of damage ($\chi^2_1 = 7.74$, $P = 0.0054$, Fig. 3a), but there was not a significant effect of common garden on severity of damage (mixed-model Poisson regression $\chi^2_{15} = 8.5$, $P = 0.90$). Leaves receiving minor damage had an average of 90 trichomes ($n = 147$, $SE = 7.7$), whereas those receiving moderate damage had an average of 63 trichomes ($n = 111$, $SE = 7.6$), and those receiving severe damage had an average of 44 trichomes ($n = 53$, $SE = 7.04$). The results shown in Fig. 2 suggest that trichome density evolves in response to the prevalence of herbivory at a given location, whereas the results in Fig. 3 suggest that trichomes may not reduce the likelihood but rather the severity of herbivory (Fig. 3a,b).

There was not a significant relationship between plant trichome density and flower production in the least-squares regression ($SS = 1.36$, $F_{1,197} = 0.025$, $P = 0.872$, Table S4), but a ‘trichome × population’ interaction term did significantly affect flower production ($SS = 1765$, $F_{14,184} = 2.3$, $P = 0.0067$, Table S4). This implies that there was variation across sites regarding the effect of trichomes on plant fitness. Of the populations carried forward for common garden experiments: trichomes were positively associated with fitness in Wildcat Creek (WC), Cascadia Santiam River (CSR), Iron Mountain (IM) and TBR and negatively associated with fitness in BR, Holden Creek (HOL), Lookout Point Dam (LPD) and Mount Washington Lookout (MWL).

Within-generation plasticity

Log-transformed trichome density of 7th leaf pairs in the glasshouse was plastic with regard to wounding of earlier leaves (GLM: $\chi^2_1 = 7.2$, $P = 0.0074$), population ($\chi^2_6 = 171.9$, $P < 0.0001$) and family nested within population ($\chi^2_{32} = 53.5$, $P = 0.01$, Table 1). Wounded plant produced on average 18.5 ($SE = 1.46$) trichomes per cross section on 7th leaves compared with 14.5 ($SE = 1.27$) by control plants. Within the populations considered, there was not a significant ‘population × damage’ effect on trichome density ($\chi^2_6 = 8.3$, $P = 0.22$, Table 1, Fig. S2). This suggests that although wounded plants generally produced a greater number of trichomes than control plants, there was no evidence for interpopulation variation in within-generation trichome plasticity. Individual population tests found wounded plants produced significantly more trichomes in WC ($\chi^2_1 = 4.46$, $P = 0.035$), TBR ($\chi^2_1 = 4.94$, $P = 0.026$) and CSR ($\chi^2_1 = 4.01$, $P = 0.043$), but not in the other populations (Fig. S2).

Transgenerational phenotypic plasticity

In both field and common garden environments, parental wounding had a significant population-dependent effect on log-transformed offspring second leaf trichome density (GLM: $\chi^2_7 = 21.96$, $P = 0.0026$, Table 2). The offspring of damaged plants from WC, HOL, TBR, CSR and BR had increased trichome density, whereas trichome production declined in the offspring of damaged plants from MWL, LPD and IM (Fig. 4a). Individual population tests find that transgenerational trichome increases were statistically significant in HOL ($\chi^2_1 = 6.92$, $P = 0.0085$) and CSR ($\chi^2_1 = 8.95$, $P = 0.0028$), but not in BR ($\chi^2_1 = 3.35$, $P = 0.067$), WC ($\chi^2_1 = 0.25$, $P = 0.62$) or TBR ($\chi^2_1 = 2.6$, $P = 0.11$) (Fig. 4a, b). Transgenerational wounding-induced trichome reductions were significant for IM ($\chi^2_1 = 6.56$, $P = 0.010$) and MWL ($\chi^2_1 = 13.8$, $P = 0.0002$), but not LPD ($\chi^2_1 = 2.2$, $P = 0.14$) (Fig. 4a,b).

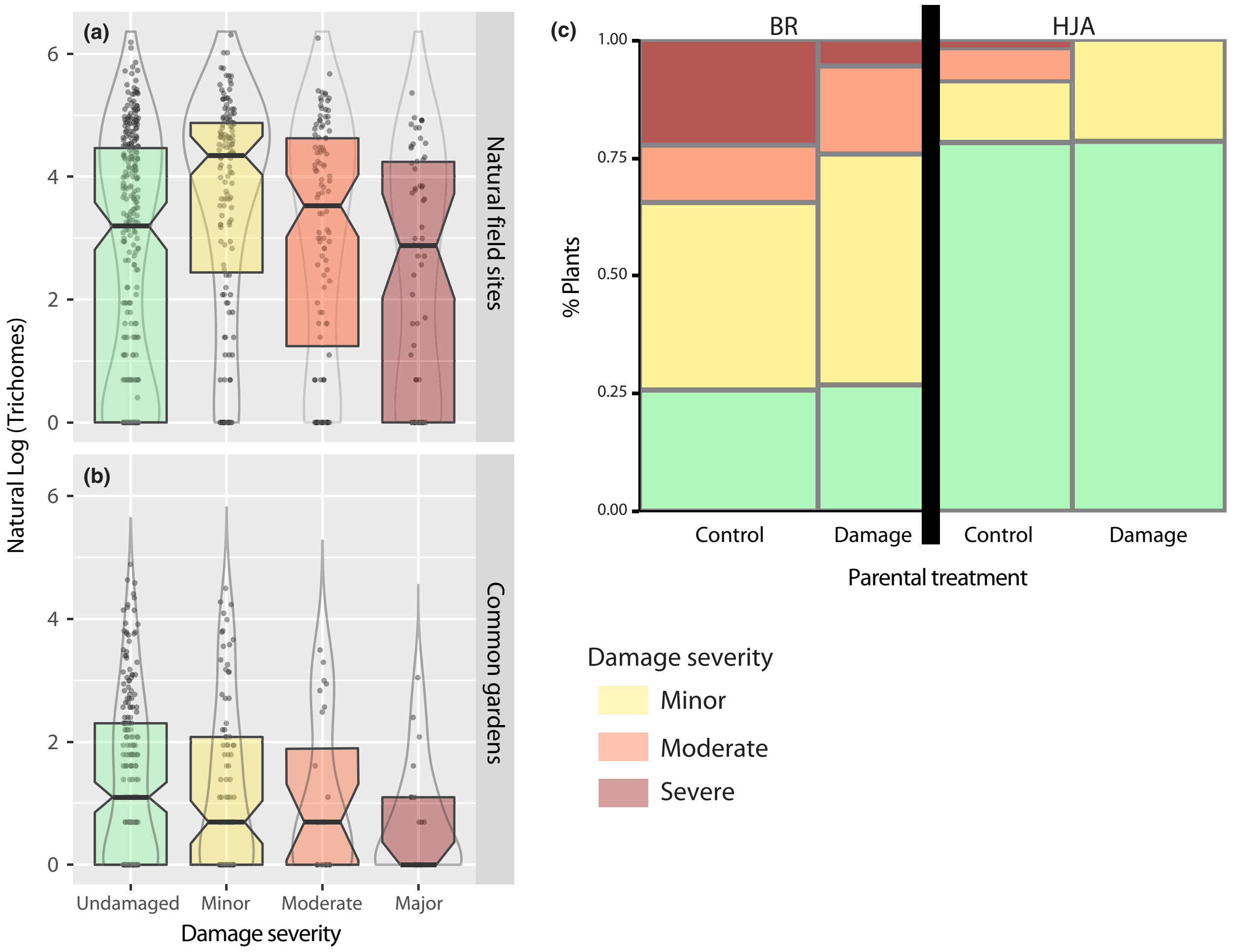

Fig. 3 The role of trichomes (a, b) and transgenerational plasticity to damage (c) in plant herbivore resistance. In both natural field sites (a) and field common gardens (b), plants experiencing minor damage tended to have more trichomes than those receiving moderate or severe damage. (c) At both HJA and Browder Ridge (BR) field common garden sites, the progeny of damaged plants experienced less severe herbivore damage than the progeny of control plants.

Table 1 Generalized linear model of trichome density accounting for within-generation plasticity to leaf wounding.

Source	d.f.	χ^2	*P*-value
Population	6	171.9	< 0.0001
Wounding	1	7.2	0.0074
Wounding × Population	6	8.3	0.2163
Family [population]	32	53.5	0.0101

Table 2 Generalized linear model of trichome density accounting for transgenerational phenotypic plasticity.

Source	d.f.	χ^2	*P*-value
Environment*	1	184.7	< 0.0001
Parental wounding	1	0.3	0.5683
Environment × parental wounding	1	0.1	0.7849
Population	7	107.1	< 0.0001
Environment × population	7	48.6	< 0.0001
Population × parental wounding	7	21.9	0.0026
Environment × population × parental wounding	7	16.2	0.0237
Family [population]	35	135.9	< 0.0001

*Environment: Field vs. Glasshouse.

Along with parental wounding, field vs. glasshouse conditions also had a large effect on trichome production ($\chi^2_1 = 184.73$, $P < 0.0001$, Table 2, Fig. 4b), with plants grown in the glasshouse producing many more trichomes than their siblings transplanted to the field. Different populations varied in the scale to which they produced more trichomes in the glasshouse (offspring environment × population, $\chi^2_7 = 48.61$, $P < 0.0001$, Table 2), but plants from all populations produced more trichomes in glasshouse compared with field environments. There was not a significant environment

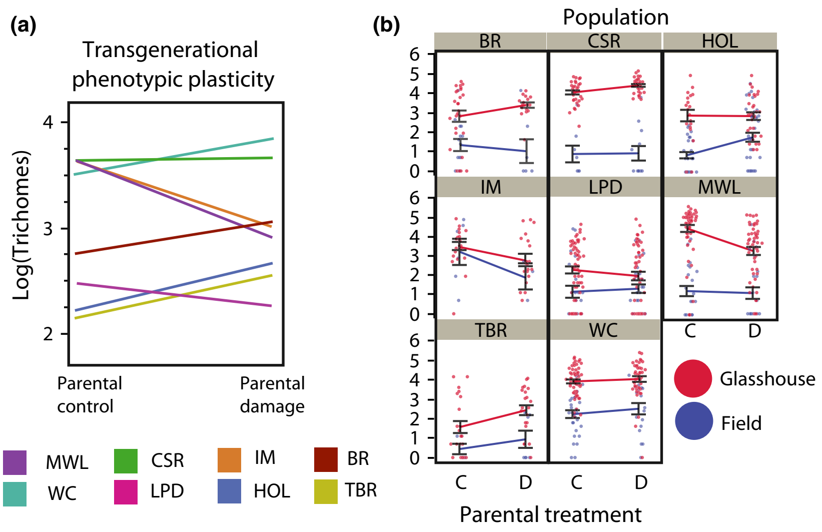

Fig. 4 Transgenerational norm of reaction for trichome density in the offspring of control and damaged parent plants across the study populations. Positive slopes represent increased trichome production in the offspring of wounded plants, and negative slopes represent decreases in trichome production. (a) Standard norm of reactions demonstrating mean population responses to parental damage. (b) Expanded norm of reactions showing population responses to parental damage, and variability of this effect across different offspring environments.

× parental wounding effect on trichome density ($\chi^2_1 = 0.074$, $P = 0.785$), but was a significant environment × parental wounding × population effect ($\chi^2_7 = 16.16$, $P = 0.024$). This three-way interaction term can be conceptualized as population-level differences in the different phenotypic outcomes of parental wounding in field vs. glasshouse offspring environments, and visualized as the differences between transgenerational reaction norms in field vs. glasshouse environments across populations (Fig. 4b).

Trichomes and transgenerational herbivore resistance

At field common garden sites, plants with a greater number of 2nd leaf pair trichomes experienced less severe herbivory than those with fewer trichomes (Poisson regression: $n = 135$, $\chi^2_1 = 5.14$, $P = 0.023$, Fig. 3b). At common garden sites, leaves that experienced no herbivory produced an average of 9.75 trichomes ($n = 217$, SE = 1.25), leaves that experienced minor damage produced an average of 9.39 ($n = 88$, SE = 1.9), moderate damage 5.80 ($n = 25$, SE = 3.5) and severe damage 2.27 ($n = 22$, SE = 3.7). There was not a significant difference in trichome density between undamaged and minor damaged leaves, or moderate and severely damaged leaves, but leaves experiencing minor or no damage produced significantly more trichomes than those experiencing moderate or severe damage ($n = 352$, $F = 5.46$, $P = 0.02$, Fig. 3b).

To further analyse the factors effecting plant herbivore damage in the field, models were constructed via both minimum BiC and AiCc to select factors impacting plant herbivore damage. Both minimum BiC- and AiCc-constructed models identified only common garden site as having a significant effect on whether or not a plant received herbivore damage ($n = 319$, BiC = 345, AiCc = 338, $\chi^2_1 = 82.8$, $P < 0.0001$, Fig. 3c); 73% of plants at BR received at least minor herbivore damage, whereas only 21% of plants at HJA experienced any herbivory.

On the other hand, our models constructed to compare the severity of herbivore damage varied greatly between minimum BiC- and AiCc-constructed models. Using a minimum BiC criterion a model, only common garden site, parental damage and their interaction term were selected as explanatory factors explaining herbivore severity ($n = 115$, BiC = 190.84, AiCc = 177.66, $\chi^2_3 = 25.29$, $P < 0.0001$, Table 3a, Fig. 3c). The offspring of damaged plants experienced less severe herbivore damage than the offspring of control plants ($\chi^2_1 = 14.8$, $P < 0.0001$, Table 3a, Fig. 3c), and plants at the BR common garden had more severe herbivory than those at HJA ($\chi^2_1 = 14.6$, $P < 0.0001$). Of the 25 offspring of damaged plants grown at HJA that experienced herbivory, all of them experienced only minor leaf damage (< 10% leaf area removal), whereas 6/19 offspring of undamaged plants at this same site experienced moderate herbivory (between 10% and 30% leaf area removal, Fig. 3c). At BR, the most striking difference was found in the severe herbivory category; only 2/27 (7.5%) offspring of damaged plants experienced severe damage (> 30% leaf area removal), whereas 13/43 (30%) offspring of control plants experienced this level of damage.

Table 3 Minimum BiC (a)- and AiCc (b)-selected models of the factors explaining herbivore damage at two field common garden sites.

Source	d.f.	χ^2	*P*-value
(a)			
Site	1	14.6	0.0001
Parental wounding	1	14.8	0.0001
Site × parental wounding	1	5.9	0.0154
(b)			
Parental wounding	1	9.7	0.0018
Pop_3 × parental wounding	1	9.4	0.0022
Pop_1	1	9.1	0.0026
Site × parental wounding	1	6.8	0.0091
Site × Pop_2	1	5.5	0.0186
Site × trichomes	1	5.3	0.0216
Site	1	5.0	0.0251
Pop_3	1	4.5	0.0341
Height	1	2.8	0.0954
Pop_2	1	0.2	0.6435
Trichomes	1	0.2	0.6969

Pop_1: LPD/WC/MWL/HOL/TBR vs. IM/BR/CSR; Pop_2: LPD/WC/MWL vs. HOL/TBR; Pop_3: LPD/WC vs. MWL.

Under the minimum AiCc-selected model, those same terms, along with eight additional terms, were found to influence herbivory levels ($n = 93$, BiC = 163.3, AiCc = 134.9, $\chi^2_{11} = 50.9$, $P < 0.0001$, Table 3b). This less conservative model included population, height, trichome density, parental damage × population, trichome × site, site × population and site × trichome × population interaction terms (Table 3b). Of particular interest, although there is a general trend of increased herbivore resistance in the offspring of damaged plants across all populations, the magnitude and direction of this response varied across populations. The offspring of plants derived from IM, BR and CSR generally received the least severe damage ($\chi^2_1 = 9.1$, $P = 0.003$, Table 3b). The offspring of damaged plants from WC showed the greatest increase in resistance, whereas the offspring of damaged plants from MWL and LPD were less resistant than the offspring of control plants ($\chi^2_1 = 9.4$, $P = 0.002$, Table 3b, Fig. S3). Of the other populations, BR, CSR and IM showed similar resistance in the offspring of control and damaged individuals, whereas HOL, LPD and TBR showed increased resistance in the offspring of damaged plants (Fig. S3).

Year-to-year autocorrelation in temperature and precipitation

Between 1895 and 2014 sites within the Oregon Cascades exhibited substantial year-to-year autocorrelations in mean annual temperature, and only minor interannual autocorrelations for precipitation. Of the four locations considered, LPD had the highest interannual autocorrelation in temperature (0.43), whereas WC had the lowest (0.22) (Fig. S4), all significantly higher than expected by chance. A second peak in temperature autocorrelations at a lag time of 8 years (Fig. S5) suggests that El Nino cycles may lead to lagged periodicity in temperature autocorrelations in the central cascades (Rasmusson & Wallace, 1983). Interannual autocorrelations in total precipitation in this region were negative in the cases of LPD (−0.15), CSR (−0.01) and MWL (−0.06), but positive at the western WC population (0.03); however, none of these autocorrelations were outside the 95% confidence interval.

Discussion

The experiments described above reiterate the role of plastic responses to the environment, genetic differentiation, and the inferred role of selection on phenotypic differentiation in plants using an extension of the common garden technique popularized by Clausen, Keck and Heisey (Fig. 1a, Clausen *et al.*, 1948). The addition of an intermediate glasshouse generation, in which plants were exposed to mechanical wounding, extends the framework to consider transgenerational effects on phenotype and plant–herbivore interactions (Fig. 1b).

In this study, both common garden and natural population surveys indicate that *M. guttatus* trichomes limit the severity of herbivory in the field (Fig. 3a,b), parental wounding alters offspring herbivore resistance (Fig. 3c) and defence phenotypes (Table 2), and that there is natural variation in these responses (Tables 2 and 3b). Previous studies in *M. guttatus* have implicated their likely role in herbivore defence (Holeski, 2007; Holeski *et al.*, 2010; Scoville *et al.*, 2011) and found their induced production coincides with the differential expression of many genes involved in plant defence (Colicchio *et al.*, 2015b), whereas other studies in *Mimulus* (Hendrick *et al.*, 2016) and elsewhere (Bickford, 2016) have also implicated trichomes in other processes. For this reason, the natural surveys of *M. guttatus* trichome variation, and tests of the relationship between trichomes and herbivore defence provided here deliver much needed support for future experimental work in this system.

Trichomes: natural variation and role in herbivore defence

Population mean trichome density is strongly correlated with population-level incidences of herbivory ($R^2 = 0.735$, Fig. 2), and at both common gardens and natural field sites, plants with more trichomes experienced less severe herbivory (Fig. 3a,b). The significant interaction between trichome density and population on flower number suggests that trichomes are related to plant fitness in a population-dependent manner

(Table S4). There are a number of potential causes for this interaction, such as correlations between trichome density and other phenotypes (Lande & Arnold, 1983), a variable role of trichomes as specialist vs. generalist herbivore deterrents (M. Rotter and L. Holeski, in review), or an interaction between nutrient availability, trichome density and plant fitness (Wilkens *et al.*, 1996). Additionally, flower number is limited as a proxy for fitness due to variation in flower size, seed set per flower, germination rates of seeds and other offspring fitness components (Reznick & Travis, 1996). Still, taken together the population-level positive correlation between trichome density and herbivore incidence, the individual-level negative correlation between trichome density and herbivore damage severity and the population-dependent relationship between trichomes and flower number suggest that *M. guttatus* trichomes play a role in herbivore defence and that there are costs associated with increased defences (Mauricio & Rausher, 1997; Mauricio, 1998) that could lead to intermediate trichome densities dependent on local herbivory.

Although our population-level analyses suggest that trichome densities may evolve in response to local herbivore prevalence (Fig. 2), the evidence here suggests that they may not function in preventing herbivory *per se*, but rather in limiting the scale of damage given that an herbivore does arrive. Trichomes did not have a significant effect on reducing the likelihood of leaves receiving herbivore damage in natural populations ($P = 0.63$, Fig. 3a), and at common garden sites undamaged leaves had similar trichome densities to those with minor damage (Fig. 3b). However, in both natural populations and field common gardens trichome density was associated with a decrease in herbivore damage severity, given that a leaf received at least minor signs of herbivory (Fig. 3a,b). Thus, it appears that whereas the frequency of herbivory selects for population trichome density levels (Fig. 2), trichomes may function to reduce the severity of damage rather than reducing the incidence of herbivory.

Unlike the hypotheses put forth under the optimal defence theory (McKey, 1974), and supported in a number of cases (Zangerl & Berenbaum, 1990; Lewinsohn *et al.*, 1991), I do not find any evidence for a trade-off between constitutive and within-generationally (Fig. S2) or transgenerationally (Fig. 4) induced defences in this study. This result reiterates findings from *M. guttatus* RILs where a similar lack of correlation between induced and constitutive defences was found (Holeski, 2007). In both cases, there is no pattern in which lines that produce constitutively higher levels of defence are any more or less plastic than their less defended relatives. Rather than herbivore abundance patterns imparting a single selective force on induced and constitutive defences, the mean and variability of such herbivore incidences over time may select somewhat independently upon constitutive, plastic and TPP-induced defences. The uncoupling of norm of reaction intercept and slope could allow organisms to adapt to their local conditions more precisely than if these two parameters were tightly linked.

Future studies that include herbivore observations will be necessary to determine whether specialist and generalist herbivores are differentially impacted by transgenerational induction, as might be expected due to the largely different manner in which plant-generalist and specialist interactions are expected to evolve (van der Meijden, 1996; Ali & Agrawal, 2012). The prevalence of TPP within the Lepidopteran herbivores that feed on *M. guttatus* in nature (Woestmann & Saastamoinen, 2016) further complicates the role of TPP in natural environments, as does the potential that complex suites of traits (Lancaster *et al.*, 2007) in *M. guttatus* are impacted by parental wounding, as suggested by the transcriptomic evidence (Colicchio *et al.*, 2015b).

Transgenerational effects on trichomes

In both glasshouse and field experiments, leaf trichome density was affected by wounding in the previous generation (Table 2). By considering TPP in multiple offspring environments, I hoped to mitigate biases due to a single beneficially or detrimentally saturated environment (Engqvist & Reinhold, 2016). Some populations showed increased trichome density in the offspring of damaged individuals, whereas plants derived from damaged LPD, MWL and IM parents produced fewer trichomes. This parental wounding × population interaction term is the most natural evidence for interpopulation variation in TPP (Sultan, 1996). Similar to Sultan (1996) and Schmitt *et al.* (1992), this study demonstrates that not only can the slope of transgenerational norms of reaction vary, but so to can the direction (Fig. 4a). This suggests that transgenerational effects could evolve to match both positive environmental autocorrelations (through positive TPP) as well as negative environmental autocorrelations (negative TPP) (Kuijper *et al.*, 2014; Kuijper & Hoyle, 2015).

Plants grown in glasshouse common gardens produced more trichomes than those that were transplanted to field conditions. Interestingly, plants collected from natural populations had a greater number of trichomes than either glasshouse or field common garden plants, a similar result to one previously described in *Cajanus cajun* (Romeis *et al.*, 1999). This result demonstrates the need to take caution when using solely glasshouse experiments as a proxy for plant defences in nature (Thaler *et al.*, 1996).

The significant offspring environment (field vs. glasshouse) × parental environment (wounded vs. control) × population effect on trichome density demonstrates that the current environment has a genotype-dependent effect on TPP (Table 3, Fig. 4b). Current environment × parental environment effects on phenotype have been observed previously (Schmitt *et al.*, 1992), and while not surprising, here I find evidence that these effects vary across populations. This somewhat curious effect demonstrates that rather than transgenerational effects providing a discrete signal to direct phenotypic change in the next generation, it acts in conjunction with genetic regulatory elements and within-generation plasticity to further alter plant development. Recent work across a diversity of plants has demonstrated that these interactions between genotype, parental environment and offspring environment are widespread and suggest that within-generation plasticity, transgenerational inheritance and genetic variation all interact and contribute to local adaptation (Sultan, 2015). In *Polygonum persicaria*, the three-way interaction between parental drought treatment, offspring germination demethylation treatment and genetic line explained over 30% of the unexplained variance in seedling biomass (Herman & Sultan, 2016). In *Arabidopsis*, recent work has highlighted the complex interactions between multigeneration exposure to salt stress, and offspring environment on a number of plant phenotypes (Groot *et al.*, 2016). Finally, the 'priming' effect, in which the offspring of stressed plants are able to rapidly induce defences in response to a similar stress, represents another known system in which this interaction between current and parental environment impacts plant phenotype (Cayuela *et al.*, 1996; Beckers & Conrath, 2007; Beckers *et al.*, 2009; Freitak *et al.*, 2009; Conrath, 2011). Future experiments aimed at discovering and analysing transgenerational effects must therefore not only carefully consider genetic variation and transgenerational environments when designing experiments, but also the offspring environments. This three-way interaction highlights the complexity of TPP and poses new questions regarding the interaction between within-generation and transgenerational plasticity.

Transgenerational effects on herbivory

Although relating parental environment with offspring phenotype has been the end goal of most transgenerational studies (Reymond *et al.*, 2000; Holeski, 2007; Colicchio *et al.*, 2015b), others have additionally considered the fitness effects of transgenerational plasticity (Galloway & Etterson, 2007; Herman *et al.*, 2012). In the plant herbivory system (Ballhorn *et al.*, 2016), recent results demonstrate that parental wounding can increase offspring resistance. Here, I corroborate that result; the offspring of wounded plants experience significantly less severe herbivory than the progeny of control individuals (Fig. 3c). Herbivory imposes strong selective pressures (Coley *et al.*, 1985), suggesting that parental wounding plays a role in altering offspring development in a way that will directly affect their fitness.

Although *M. guttatus* trichomes appear to evolve according to the probability that a plant will experience herbivory (Fig. 2), and slightly reduce the probability that a plant will be damaged (Fig. 3b), they also reduce the severity of damage after herbivore arrival (Fig. 3a, b). Future studies that include herbivore monitoring will be necessary to elucidate the precise stage at which trichomes function to reduce or avoid herbivore damage.

This distinction between traits that allow plants to resist herbivore damage, avoid herbivore damage and tolerate herbivore damage represents distinct but non-mutually exclusive mechanisms that allow plants to cope with herbivores (Strauss & Agrawal, 1999; Tiffin, 2000), and parallels the diversity of mechanisms (tolerance, avoidance, escape) that plants employ to deal with drought (Kooyers, 2015) or freezing (Sakai & Larcher, 2012). Considering adaptation more generally, (i) escaping stress before it limits fitness, (ii) minimizing initial stress to reduce concomitant fitness loss and (iii) mitigating the long-term detrimental effects of stress, presents a chronological and intuitive method to parameterize how traits mediate adaptation in the face of stress. Here, we model the possible role of trichomes or general parental wounding effects via mechanisms (i) and (ii), but future experiments utilizing herbivore manipulations will be necessary to test the role of herbivory tolerance (iii) in this system. Natural genetic variation in the fitness impacts of herbivory has been demonstrated (Strauss & Agrawal, 1999; Więski & Pennings, 2014), but to this point no one has tested the possibility that parental wounding may impact this relationship.

In much the same way that there was a population × parental wounding effect on trichome production, this same interaction term also significantly affected herbivore resistance (Table 3b, Fig. S3). Although the two components of this finding have been previously demonstrated, transgenerational effects alter plant herbivore resistance in field conditions (Ballhorn *et al.*, 2016) and TPP varies across genotypes (Galloway & Etterson, 2009), this is the first evidence that TPP for herbivore resistance varies across populations. Considered alongside theory regarding the evolution of TPP in different environments (Kuijper & Hoyle, 2015; Uller *et al.*, 2015), this result suggests that the natural variation in TPP exists upon which environmental patterns could act to increase or decrease the relative importance and direction of transgenerational plasticity.

Although herbivores in other locations exhibit population dynamics that lead to interannual autocorrelations in their density (Turchin, 1990; Klapwijk *et al.*, 2013), future studies will be necessary to test for interannual autocorrelations in herbivory and TPP responses in the same system. Alternatively, natural variation in

TPP could be due variable spatial heterogeneity in herbivory, similar to its hypothesized role in life history shifts due to parental shading (Galloway & Etterson, 2007). If the location of a parent plant within a population is correlated with the herbivore wounding severity the offspring are likely to receive, the evolution of TPP may also be expected (Herman *et al.*, 2014).

One interesting dichotomy between spatial autocorrelation- and temporal autocorrelation-driven TPP is that in the case of spatial heterogeneity, maternal environment is likely to contain higher quality information than paternal information and thus lead to the evolution of maternal effects (Fox & Mousseau, 1998). However, if transgenerational plasticity evolves in response to patterns of temporal autocorrelation, both maternal and paternal environment will contain information of similar value, and thus, the integration of paternal and maternal effects would likely prove adaptive (although explicit theory and modelling will be needed to consider both spatial and temporal autocorrelations in tandem). In *M. guttatus,* both paternal and maternal wounding is known to induce trichome production to a similar scale (Akkerman *et al.*, 2016), but 5-azacytadine experiments demonstrate that the molecular mechanism is distinct between the maternally and paternally inherited signals. This suggests that maternal and paternal effects may evolve separately, and at least in a single RIL, the nearly equal dual inheritance is indicative of the flavour of TPP that is expected to evolve in response to temporal rather than spatial autocorrelations.

Natural variation in TPP to herbivory

Considering interpopulation variation in TPP for trichome production (Fig. 4a,b, Table 2) alongside variation for TPP in herbivore resistance (Table 3, Fig. S3), there is substantial support for natural variation in TPP within these populations of *M. guttatus*. In five of the eight populations, there was support for TPP of induced herbivore resistance that is in part mediated through increased trichome production (Fig. 3a). Within LPD, there is evidence of induced herbivore resistance, but not trichome production, potentially implicating other forms of defence, such as leaf PPG synthesis (Holeski *et al.*, 2013) in the transgenerational response. Seed herbivory in the developing fruit is extremely common at LPD. As leaf trichomes have no clear effect on seed herbivory, it is possible that floral defence traits rather than vegetative traits, such as increased floral tissue thickness (Roubik, 1982) or calyx trichomes may be more relevant to local adaptation to herbivory at this site.

Peculiarly, in MWL there is evidence of negative transgenerational induction (reduced trichome production and herbivore resistance in the progeny of damaged plants, Figs 4b and S2), with moderate support for negative induction in IM (reduced trichome density but no change in herbivore resistance, Figs 4b and S2). Of the eight populations considered, the two that showed signs of negative transgenerational induction, IM and MWL, were derived from the highest elevation and furthest north sites (Table S1). However, IM is located only 8 miles from BR, which, along with the next most northern population, CSR, both show signs of positive transgenerational plasticity. Additionally, IM and BR share a great deal of common genetic variation (average pairwise $F_{ST} = 0.065$) (Monnahan *et al.*, 2015), suggesting that neutral divergence is unlikely to account for the observed differences in TPP. Still, it could be that IM and MWL share a common ancestry relative to the rest of the populations and that drift processes rather than selection have led to negative TPP arising to high frequencies within those populations. Alternatively, being derived from high elevation and latitude, it could be that a certain aspect of one, or both, of these variables generates patterns of interannual herbivore abundance that favours negative TPP.

Potential role of climatic autocorrelations in TPP evolution

Although long-term herbivory data are not available at these locations, climate data from 1895 to 2014 in the central Cascades does exhibit significant patterns of year-to-year autocorrelation for annual mean temperature, but not precipitation. Both temperature and moisture availability impact herbivore activity (Bale *et al.*, 2002), so these climatic variables should relate to patterns of interannual herbivore variation. At all four locations, there were positive autocorrelations in mean annual temperature, with autocorrelation values varying from 0.22 to 0.43 (Fig. S4). As mean annual temperature influences herbivore activity (Lemoine *et al.*, 2014), the observed pattern of temperature autocorrelation may give rise to positive autocorrelations in herbivore abundance, in turn selecting for genotypes that transmit herbivore-induced defences between generations. Long-term studies of insect densities (Turchin, 1990) and more recently insect herbivore damage (Klapwijk *et al.*, 2013) confirm that herbivore densities between consecutive years tend to be positively autocorrelated. While the direct relationship between climatic autocorrelations and biotic autocorrelations, such as herbivore abundance, is unclear (Jactel *et al.*, 2012), and for some climatic parameters appears nonexistent (Swanson, 1998), there is evidence that underlying abiotic autocorrelations can select for the evolution of transgenerational effects (Petchey, 2000; Dey *et al.*, 2016).

While high temperatures during the growing season should increase herbivore activity in the short term, it also reduces snowpack going into winter (Walker *et al.*, 1999; Pederson *et al.*, 2011). During the following growing season, decreased snowpack will reduce moisture availability (Luus *et al.*, 2013). Thus, while at high elevations interannual temperature autocorrelations exist, the relationship between temperature and snowpack may generate a negative autocorrelation in interannual herbivore activity. Low elevation sites are less arid (Table S1), and moisture availability is not as heavily reliant on snow melt (personal observations); therefore, this negative autocorrelation is not expected to be as prominent. This elevation-dependent relationship between temperature and moisture availability, and the further complex relationship of drought conditions on herbivore activity (Jactel *et al.*, 2012) may explain the presence of positive transgenerational trichome induction and herbivore resistance at low but not high elevation sites; however, this prediction remains to be tested. Supporting the possibility that elevation, latitude or growing season length may in some way drive differences in TPP to herbivory in *M. guttatus*, previous studies demonstrated that trichome induction is prevalent in individuals derived from low elevation Point Reyes coastal perennial plants, but not in high elevation derived IM plants (Holeski, 2007).

Conclusions

The optimal allocation of resources towards defence traits will evolve to an equilibrium based on the costs and benefits of this defence (McKey, 1974; Caswell & Reed, 1976). This study provides evidence that along with natural variation in constitutive and inducible defences (Lewinsohn *et al.*, 1991; Moreira *et al.*, 2014), natural variation in TPP can also impact plant defence in nature. This variation in plant abilities to integrate environmental information between individuals across space, through volatile compounds (Kessler & Baldwin, 2001) and time (shown here) begins to reveal the complexity of plant–plant communication. Interplant communication through TPP and volatile compound communication (Kessler & Baldwin, 2001) both serve to increase the information available to an individual, and in turn increase their ability to produce optimal phenotypes under a given environment. In this light, genetic regulation, plastic responses and interindividual communication act in the same vein to maximize the mutual information between organism phenotype and selective environment (Frank, 2009; English *et al.*, 2015).

While the contribution of transgenerational effects on fitness in nature has numerous ecological implications, the evolutionary significance of TPP depends upon genetic variation for transgenerational plasticity. This study demonstrates the presence of interpopulation variation for TPP (Fig. 4a,b), which, alongside evidence of within population variation for TPP (Galloway & Etterson, 2007), suggests that local environmental patterns of variation can favour genotypes with different capacity to transmit information between generations. *Mimulus guttatus* trichome induction can be stable across two generations (grandmaternal effects) (Akkerman *et al.*, 2016), bringing up the possibility that there is variation not only in the TPP signal across a single generation, but also in its persistence (Prizak *et al.*, 2014). It could be that populations strongly affected by long-term climatic oscillations such as El Nino may select for the multigeneration stability of TPP. The persistence of *M. guttatus* trichome induction across at least two generations suggests that future studies considering interannual autocorrelations in herbivory and TPP along altitudinal, latitudinal and climatic autocorrelation clines will be necessary to test the different possibilities presented above. In this study, the use of a replicated circular crossing design in the parental generation was used to limit the confounding effects of multigeneration TPP by ensuring that the progeny of damaged and control plants derived from the same grandparents, but grandparental effects could be responsible for some of the observed mean trichome density differences between populations.

One possible explanation for this system of inherited environmental information is that a portion of the environmentally induced epigenetic changes (such as DNA methylation or histone modifications) are not reset, but rather passed between generations (Verhoeven *et al.*, 2010). Further work is necessary to determine the mechanism through which epigenetic effects are reiterated in the germ line, but evidence for the epigenetic basis of TPP is mounting. Methylation changes in response to environmental stress (Wang *et al.*, 2010; Dowen *et al.*, 2012), stably transmitted epigenetic markings (Rasmann *et al.*, 2012; Slaughter *et al.*, 2012; Schmitz *et al.*, 2013; Li *et al.*, 2014) and epigenetic effects on gene expression (Colicchio *et al.*, 2015a) all point towards epigenetic inheritance as the source of TPP. Additionally, recent work has demonstrated that the erasure of DNA methylation markers reduces or completely erases TPP to wounding (Akkerman *et al.*, 2016) and drought stress (Herman & Sultan, 2016). Additionally, maternal effects due to seed nutrient allocation, morphology or coating (Galloway, 2001; Luzuriaga *et al.*, 2006) could alter offspring defences through currently unknown mechanisms.

Adaptive TPP in nature was first demonstrated to play a role in the transition between annual and biennial life history strategies in response to maternal light conditions in *Campanulastrum americanum* (Galloway & Etterson, 2007, 2009). Here, utilizing the system of plant herbivore defence, this work gets expanded upon by considering natural TPP variation across multiple populations, measures of offspring fitness and phenotype in field common gardens, and patterns of interannual autocorrelation of environmental factors. Future experiments considering the molecular variation in TPP across populations and species, direct measures of interannual herbivory variation and plant secondary metabolite phenotypes in conjunction with trichome density will be necessary to further increase our understanding of how transgenerational plasticity alters biotic interactions in nature.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Diagnostic plots regarding the results of the GLM used to explain natural variation in plant Log(Trichome Density).

Figure S2 (a) Within generation norm of reaction of mean population responses to early leaf wounding on seventh leaf trichome density. (b) Norms of reaction of mean responses for each population, with individual plants as data points and standard error bars.

Figure S3 Mosaic plot of the severity of damage received by plants from the eight experimental populations at the two field common gardens.

Figure S4 Annual temperature and mean precipitation between 1895 and 2014.

Figure S5 Inter-annual mean temperature autocorrelations at Iron Mountain.

Table S1 Sixteen natural populations studied in this experiment.

Table S2 Model predicting mean population trichome density at 16 populations surveyed for this experiment.

Table S3 Model of various climatic and geographical factors explaining percent leaf herbivory at natural populations.

Table S4 ANOVA of factors influencing flower production at 15 natural populations.

Data deposited at Dryad: doi: 10.5061/dryad.89r82

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