Variation in growth and defence traits among plant populations at different elevations: implications for adaptation to climate change

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ABSTRACT

1. Alpine plants occurring at high elevation are vulnerable to ongoing climate change, yet relatively little is known about the potential for high-elevation species to adapt to changing environmental conditions. In particular, the extent to which high-elevation plants will be able to resist predicted increases in the intensity of biotic interactions, such as herbivory, remains unclear.

2. Species distributed across broad elevational ranges provide an opportunity to investigate evolutionary mechanisms and traits involved in adaptation to varying abiotic and biotic environments. This study focused on the perennial alpine plant *Arabis alpina* and combined field surveys and climate-chamber experiments to test for intraspecific genetic divergence in traits related to growth and defence against herbivores. We screened multiple populations from low, intermediate and high elevations across a broad geographic area, characterising differences in growth form, leaf structural traits, palatability for herbivores and defensive chemistry. We then quantified the proportion of variation explained by elevation and population-level effects.

3. Our results document within-species genetic divergence in multiple traits relevant for adaptation to the different abiotic and biotic pressures experienced at low and high elevations. Rates of herbivore damage declined with increasing elevation in the field, but plants from high- and intermediate-elevation populations were generally more palatable for specialist herbivores than those from low-elevation populations in feeding assays. Elevational clines were also observed in several glucosinolate defence compounds, and leaf herbivory more strongly induced glucosinolates in plants from high-elevation populations than in those from low-elevation populations. Leaf trichome density and growth form also diverged among populations contributing to growth-defence phenotypes associated with different elevations.

4. However, populations from similar elevations often differed significantly in both growth and defence-related traits, with trait variation often better explained by population-level effects than by elevation alone.

5. Synthesis: *Arabis alpina* exhibits patterns of genetic variation in growth and defence traits consistent with adaptation to different elevations. However, populations from similar elevations also diverged in many of these ecologically relevant traits. Together, the extent of the observed trait variation suggests that this alpine species has considerable potential to adapt to a changing biotic environment.

KEYWORDS

herbivore, alpine, environmental change, elevation, defense, growth, glucosinolate, *Arabis alpina*

INTRODUCTION

Despite increasing evidence that climate change is affecting the composition of local communities and altering interactions between species (e.g. Walther, Post et al. 2002, Pauli, Gottfried et al. 2012, Rasmann and Pellissier 2015), our understanding of the capacity for species to adapt to resulting changes in the frequency or intensity of biotic interactions remains limited (Lavergne, Mouquet et al. 2010, Hoffmann and Sgro 2011, Urban, Bocedi et al. 2016). One well-established approach to investigating species' adaptive potential entails studying populations distributed along spatial environmental gradients (De Frenne, Graae et al. 2013, Urban, Bocedi et al. 2016). Furthermore, because the intensity of biotic interactions is predicted to decline with increasing elevation (Körner 2007, Rasmann, Pellissier et al. 2014), as well as latitude (Schemske, Mittelbach et al. 2009, De Frenne, Graae et al. 2013, Anstett, Nunes et al. 2016), species distributed along such gradients

provide promising systems for studying adaptation to varying biotic pressures (De Frenne, Graae et al. 2013, Helsen, Acharya et al. 2017). To date, however, the extent to which variation in traits relevant for adaptive responses to novel or changing biotic interactions is predictably distributed along such gradients remains unclear.

Alpine environments hold particular promise for exploring such questions, as they are characterised by large changes in elevation and associated environmental conditions over relatively short geographic distances (Rasmann, Pellissier et al. 2014, Moreira, Petry et al. 2018). In addition, high-elevation plant communities are thought to be particularly vulnerable to biotic challenges associated with climatic change (Walther, Post et al. 2002, Körner 2003), including increasing competition due to upward shifts of previously low-elevation species (Pauli, Gottfried et al. 2012, Alexander, Diez et al. 2015, Rumpf, Hulber et al. 2018) and more frequent or novel interactions with invertebrate herbivores (Rasmann and Pellissier 2015). Because high-elevation species are often unable to disperse to more suitable (i.e., even higher elevation) environments, they often must adapt to such changes *in situ* or suffer significant population declines (e.g. Cotto, Wessely et al. 2017). However, we currently have limited empirical data regarding the potential for high-elevation plants to adapt to the predicted biotic challenges.

Invertebrate herbivores represent an important and well-studied class of biotic plant antagonists, and a growing number of studies have examined variation in plant-herbivore interactions along elevation gradients (Rasmann, Pellissier et al. 2014). Most such studies have reported decreasing rates of herbivory with increasing elevation, giving rise to the prediction of corresponding elevational trends in plant defence investment (Rasmann, Pellissier et al. 2014, Moreira, Petry et al. 2018). Consistent with this prediction, several studies have found that plants from higher elevations are more palatable to generalist herbivores than those from lower elevations (Ereli, Ayres et al. 1998, Pellissier, Fiedler et al. 2012, Callis-Duehl, Vittoz et al. 2016, Descombes, Marchon et al. 2017). Moreover,

constitutive chemical and morphological defences have been observed to decline with increasing elevation (Løe, Toräng et al. 2007, Pellissier, Roger et al. 2014, Rasmann, Buri et al. 2014, Zhang, Tonsor et al. 2015). However, a recent review by Moreira *et al.* (2018) highlighted a significant number of studies showing increasing defence investment with elevation (Koptur 1985, Rasmann, Pellissier et al. 2014, Abdala-Roberts, Rasmann et al. 2016, De Long, Sundqvist et al. 2016, Buckley, Pashalidou et al. 2019), as well as other studies reporting no or non-linear associations with elevation (Louda and Rodman 1983, Rasmann, Pellissier et al. 2014, Dostalek, Rokaya et al. 2016). Furthermore, several recent studies have shown that different defensive strategies, including tolerance and constitutive and induced defences, can exhibit contrasting elevational gradients (Abdala-Roberts, Rasmann et al. 2016, Dostalek, Rokaya et al. 2016, Pellissier, Moreira et al. 2016, Defossez, Pellissier et al. 2018). Such contrasting elevational trends in defence traits may partly reflect variation in herbivore pressure among species and populations that is itself independent of elevation (Moreira, Petry et al. 2018), but also suggest that herbivore pressure alone is often insufficient to explain variation in defence investment. Instead, adaptation to varying intensities of abiotic factors along elevation gradients may give rise to variation in plant defence investment that is independent of, or oppositional to, trends predicted by elevation alone (e.g. Abdala-Roberts, Rasmann et al. 2016, Pellissier, Moreira et al. 2016, Galmán, Abdala-Roberts et al. 2018). It is therefore important to consider both the biotic and abiotic selective forces that can shape patterns of defence investment along elevation gradients.

Adaptive traits that help plants cope with harsh abiotic conditions at high elevations may also indirectly influence their ability to defend themselves against herbivores. For example, higher leaf trichome densities can increase plant resistance to UV-B radiation or arid conditions (Kessler, Siorak et al. 2007, Yan, Pan et al. 2012), which may be adaptive at high elevations, but could negatively impact herbivore feeding. On the other hand, plants at

104 higher elevations tend to exhibit reduced size and lower specific leaf area than those at
105 lower elevations, which might facilitate survival under harsh abiotic conditions (Körner,
106 Neumayer et al. 1989, Byars, Papst et al. 2007, Bello, Lavorel et al. 2013, Read, Moorhead
107 et al. 2014, Halbritter, Fior et al. 2018), but in this case the effects on herbivores is not
108 clear. Furthermore, declining resource availability with increasing elevation may impose
109 more stringent trade-offs between investment in growth and defence (Coley, Bryant et al.
110 1985, Herms and Mattson 1992, Hahn and Maron 2016). The interacting effects of these
111 and other abiotic and biotic selective pressures across different elevations (Kergunteuil,
112 Descombes et al. 2018) may explain the existence of growth-defence "syndromes"
113 characteristic of species occurring at similar elevations (Defossez, Pellissier et al. 2018,
114 Kergunteuil, Descombes et al. 2018, Moreira, Petry et al. 2018). Consequently, to
115 understand the complex selective factors shaping elevational variation in defence
116 investment, it is necessary to assess variation in multiple growth and defence traits along
117 broad elevational gradients.

118 In addition, assessing the potential for evolutionary change in these traits requires
119 determining whether observed phenotypic variation has a genetic basis. Genetic
120 contributions can be quantified via common-garden experiments, either in the greenhouse
121 or field, and studies employing this approach have documented within-species genetic
122 variation in defence traits distributed along elevation gradients (Garibaldi, Kitzberger et al.
123 2011, Anderson, Perera et al. 2015, Pellissier, Moreira et al. 2016, Rokaya, Dostálek et al.
124 2016). However, these studies have typically compared plants from pooled sets of high-
125 and low-elevation populations, making it impossible to assess genetic variation among
126 populations from similar elevations. Meanwhile, a handful of common-garden studies have
127 screened population-level variation in chemical defence expression along elevation
128 gradients (Dostalek, Rokaya et al. 2016, Rokaya, Dostálek et al. 2016), but these did not
129 explicitly quantify the amounts of trait variation explained by population-level and

elevational effects. Indeed, while both elevation-driven and population-level effects on trait variation are important for understanding potential adaptive responses to biotic change, we are unaware of any previous study that estimated the relative contribution of each to variation in defence traits.

In the current study, we sampled populations across the elevational range of the short-lived perennial alpine plant *Arabis alpina* (Brassicaceae) in Switzerland and tested for genetic variation in several traits related to leaf structure, growth and defence, which were selected because of their potential importance for resisting or tolerating herbivory. After assessing elevational trends in rates of herbivore damage in the field, we grew plants from different populations in a common garden to test for genetic variation in our selected traits. Specifically, we tested whether specialist invertebrate herbivores performed better on high-elevation populations than on low-elevation populations, and whether growth and defensive traits differ among populations from different elevations under controlled growth chamber conditions. We then quantified the relative effects of elevation and population on variation in these different growth and defence traits. In addition, we used these data to explore whether high-elevation *A. alpina* populations exhibit consistent trait combinations that may influence their potential to adapt to increasing rates of herbivory predicted with ongoing climate warming.

MATERIAL AND METHODS

Study system background: *Arabis alpina* (Brassicaceae)

Arabis alpina is a short-lived perennial species with a wide geographic distribution in alpine environments across Europe, having colonised the Alps from multiple Mediterranean refugia following the last glacial period (Koch, Kiefer et al. 2006, Ansell, Stenoien et al. 2011, Rogivue, Graf et al. 2017). Despite its emergence as a model perennial species for studying the genetic basis of variation in flowering time and the transition to

156 selfing (Bergonzi, Albani et al. 2013, Tedder, Carleial et al. 2015), relatively little is known
157 about its interactions with natural herbivores and traits involved in adaptation to different
158 elevations. Long-range reciprocal transplant experiments between Sweden and Spain have
159 shown differential survival and reproductive effort consistent with local adaptation
160 (Törang, Wunder et al. 2015). More recent studies involving transplants across different
161 elevations at a finer spatial scale also found evidence for local adaptation, as well as strong
162 plasticity in reproductive and growth traits (de Villemereuil, Mouterde et al. 2018).
163
164 **Field surveys of plant growth form and herbivore damage**
165 In the Summer of 2016, *Arabis alpina* populations at 19 field sites distributed across
166 the Swiss Alps were surveyed for variation in leaf damage by herbivores (Table S1; Fig
167 1a). Visits were timed to coincide with the ripening of fruits, in order to simultaneously
168 collect data on cumulative leaf damage and collect seeds for use in subsequent experiments
169 (numbers sampled given in Table S2). The field sites were distributed from 797m to 2866m
170 above sea level and were visited between 23rd June 2016 and 4th Sept 2016.
171 *A. alpina* populations at these field sites exist as a set of fragmented patches of
172 plants. To avoid sampling related plants, we ran a transect through multiple patches per
173 population, with a minimum distance of 2m between surveyed plants in a patch and a
174 greater distance (tens of metres) between patches. Dispersal distances of up to 1km have
175 been estimated for *A. alpina* using genetic markers, although just over a third of offspring
176 were recorded less than 5m from a parental plant (Buehler, Graf et al. 2012). It is therefore
177 possible some related plants have been sampled in the current study, but by sampling
178 broadly across sites we minimised our sampling of related individuals. A small quadrat (18
179 x 18cm) was placed over each surveyed plant, and the surface area occupied by *A. alpina*
180 was recorded (a measure of plant size). Depending on local population size and plant
181 accessibility, 7-27 plants per population (in total 316 plants; Table S2) were haphazardly

chosen along the transect for assessment of leaf herbivore damage. The total number of leaves and the number of damaged leaves were recorded. We based our damage estimates on the number of leaves damaged rather than percentage leaf area removed, as the compact rosettes and numerous small leaves of *A. alpina* made it challenging to accurately assess the latter metric in the field. Additionally, we noted the presence of different types of leaf damage on a patch (leaf holes, chewed edges, larval trails and pale spots; see Figure 1a and Figure S1 for photos). Finally, ripe fruits were collected in small paper envelopes and stored at room temperature in the dark until seeds were used in germination experiments. Plants derived from one maternal plant in the field are hereafter referred to as a maternal family. For populations AalN2 and Aal20, fruits collected from the field in 2015 were used.

We tested the effects of population and elevation (metres above sea-level) on the different response variables in separate statistical models. Variation in number of leaves per plant and in leaf size was analysed using Generalised Linear Models (GLMs), with poisson and normal error distributions respectively, using R statistical software (R Development Core Team 2012). The proportion of damaged leaves and the presence or absence of the four different types of damage were analysed using binomial GLMs. The significance of population and elevation effects was tested by removing each factor from its respective model and assessing the significance of the change in model explanatory power using likelihood ratio tests. For each model, we estimated the proportion of variance explained by either population or elevation in the model.

To explore whether geographic or climatic factors might explain elevational trends in the average proportion of leaves damaged (following arc-sine transformation), we conducted a linear regression using four explanatory factors: decimal degrees latitude, decimal degrees longitude, average annual temperature (1961-1990) and the average sum of annual precipitation (1961-1990). Data for the two climatic factors were estimated at a 25m resolution for each population (Zimmermann and Kienast 1999). If a significant

elevation effect disappears when controlling for climatic variables, it suggests that those variables, rather than elevation *per se*, explains variation in rates of herbivory (Abdala-Roberts, Rasmann et al. 2016, Galmán, Abdala-Roberts et al. 2018).

Assessment of variation in growth-related traits, leaf structural traits and plant defensive traits in a common environment

Experiment 1: Assessing variation in growth-related traits and herbivore performance

Ripe seeds from 8 maternal families from each of the 16 study populations (representing 123 families in total) were germinated in 54-cell trays filled with pre-watered low nutrient soil (Alpine wildflower soil mix, see Supplementary Information for composition). Five seeds per family were placed 2-3mm below the soil surface in a cell, and families and populations were randomised across trays. The trays were stratified for 8 days at 4ºC (8hrs:16hrs, light: dark) to synchronise germination, before being moved to a climate chamber set to 23ºC: 17ºC, 12hr light (15kLux): 12hr dark (0kLux). After most seeds had germinated, temperatures were reduced to 18ºC (light) and 15°C (dark) for the remainder of the experiment. After 3 weeks, one seedling per maternal family was individually transferred to a 5cm pot filled with the same soil mix. Pots were randomly positioned in the growth chamber and watered 3 times per week by hand. Seedlings remaining in the tray were thinned to leave one seedling per cell. These remaining seedlings were harvested to measure dry aboveground mass approximately 42 days after seeds were moved to germination conditions. The aboveground parts were dried at 65 ºC for 2 days and then weighed on a balance to the nearest 0.001g (Mettler AE240, Mettler Toledo, Greifensee, Switzerland). The length of the longest leaf of the remaining plants was measured to the nearest millimetre about 49 days after seeds were moved to germination conditions. Maximum leaf length was used as a proxy for rosette diameter,

which is difficult to measure in a standardised manner beyond the earliest growth stages in this species.

Three populations from each of the three elevation classes (low: <1600m above sea level; intermediate: 1600-2300m; high: >2300-3000m) were then used for larval performance assays at the temperatures described previously (18ºC/15°C). This allowed us to estimate the variance explained by population and elevation class. Five first-instar *Pieris brassicae* larvae, from a lab colony reared on brussels sprout plants (*Brassica oleracea*), were added to each plant. The larvae were individually weighed after 8 days to the nearest 0.001mg on a balance (Mettler Toledo MT5). General linear mixed effects models (lme4 R package; (Bates, Maechler et al. 2014) were constructed using either population or elevation class as a fixed effect, and a random effect of individual plant. Log transformations were used, where necessary, to improve model fit based on inspections of model residuals.

Experiment 2: Assessing variation in growth-related traits, leaf structural traits and chemical defence induction

We conducted a separate experiment with the same nine populations to explore variation in a greater number of morphological and growth traits, as well as variation in defence induction. Due to limited growth-chamber space, plants were grown in a greenhouse under slightly warmer conditions than those used in the previous experiment (20ºC: 17 ºC light: dark regime). Seeds from 10 maternal plants per population were germinated as described above, with seedlings then transplanted into 7cm clay pots and allowed to grow to two months of age. To identify traits that might explain variation in herbivore performance among populations, we added three first-instar *P. brassicae* larvae to each of the plants. After 6 days of feeding, larvae were weighed to the nearest 0.001mg. Due to space limitations, plants were divided into two experimental sets (5 genotypes per population per

set) for the larval performance assay, and assays on the two sets were conducted one week apart in the same chamber. After larval weighing, we measured maximum leaf lengths and the number of leaves greater than 0.5cm length (as a proxy for investment in leaf production). The number of leaves showing any sign of damage and the number showing more than 25% leaf area removed were also counted to assess variation in plant palatability. We also measured specific leaf area (SLA) and trichome density, as these traits may impact rates of herbivore feeding. Two 6mm diameter leaf discs were cut from each of two fully expanded leaves per experimental plant, avoiding the main leaf vein. Leaf discs were dried for 48hrs at 50°C and then weighed to the nearest 0.001mg to estimate specific leaf area (leaf disc area divided by dry mass). Trichomes were counted, using a cell counter plugin in the ImageJ software program (Schneider, Rasband et al. 2012), on one lower leaf disc per plant photographed using a microscope (Leica M420) and camera (Leica MC170 HD, Leica microsystems, Wetzlar, Germany).

Variation among populations in average larval mass per plant (based on those alive at the end of the experiment) was regressed against variation in SLA, trichome density, leaf number and length of the longest leaf in a full linear model. The response variable was log-transformed to improve model fit following inspection of the distribution of residuals. Experimental set was included as a fixed term in the model, and the effect of each variable tested sequentially removing non-significant terms from the full model.

Separate GLMs with normal error distribution were used to test for effects of elevation class or population on maximum leaf length and seedling dry mass across all 17 populations in experiment 1 and for maximum leaf length and trichome density in experiment 2. Log transformations were used, where necessary, to improve model fit based on inspection of model residuals. In experiment 2, the effect of elevation class and population on leaf number was analysed using a GLM with quasipoisson error (the model was overdispersed using just poisson error). Finally, variation in log-transformed SLA was

tested using a general linear mixed model with a random effect of plant genotype. The significance of the effect of elevation class or population was tested by removing the factor and comparing the change in model likelihood to the null model.

To examine whether particular combinations of morphological and growth traits were associated with different elevations, we also conducted a Principal Components Analysis using measurements of longest leaf length, leaf number, trichome density and SLA for each plant in experiment 2. The first two principal components, and the loadings for each trait, were plotted to visualise trait divergence among plants from low, intermediate and high elevations.

After being weighed, larvae were returned to each plant for 24hrs, and six plant genotypes from each of six populations (two low, two intermediate, and two high) were selected for screening of glucosinolate induction. Each genotype was represented by two individual plants: one used for herbivore induction and one control. Replication was therefore at the level of genotype for each population. Glucosinolates are expected to be a key chemical defence in *A. alpina*, as they are for many Brassicaceae species, but to our knowledge this species has not previously been screened for glucosinolate variation (Windsor, Reichelt et al. 2005). Two leaves from each induced and control plant were weighed, immediately frozen in liquid nitrogen and then stored at -80°C. Glucosinolate extractions were performed as described in a recent HPLC protocol (Grosser and van Dam 2017), but with minor modifications. Columns were prepared using DEAE Sephadex A25 (Sigma-Aldrich, St. Louis, Missouri, US). Leaves were freeze-dried and ground to a fine powder for 1 min at 1500rpm in a Geno/Grinder 2010 (SPEX sample prep, Metuchen, NJ, US) with three 0.3mm steel grinding balls. Samples were suspended in 1mL 70% methanol and heated to 85ºC for 15mins to denature the myrosinase enzyme. Following elution of samples incubated overnight with sulfatase, samples were dried down on a Savant Speed Vac Concentrator SPP1010 (Thermo Scientific, Reinach, Switzerland) and re-suspended in

311 150μl ultrapure MilliQ water (Merck, Darmstadt, Germany). Samples were run on an
312 Agilent 6550 iFunnel Q-TOF LC/MS equipped with an Eclipse XDB-C18 column (4.6 x
313 150mm, 5µm, 80Å) using a water (with 5mM ammonium formate) to acetonitrile gradient.
314 The mobile phase conditions were as described by Grosser & van Dam (2017) and
315 consisted of 98% water for 2 minutes, then a gradient to 65% water over 35 minutes,
316 followed by a rapid gradient to 2% water over 8 minutes. Where possible, desulfo-
317 glucosinolates were identified using known laboratory standards (progoitrin, gluconapin
318 and glucobrassicanapin). Alternatively, identification of putative desulfo-gluosinolates was
319 based on the fragmentation pattern due to the loss of a hexose-derivative from a parent
320 aglycone, demonstrated by a mass shift of 162 amu, and through formula matches
321 identified using Agilent MassHunter qualitative software. The integration of the 229 nm
322 UV spectrum was used for quantification of compounds based on a comparison to a
323 sinigrin concentration curve and published response factors (again as described in Grosser
324 & van Dam, 2017). Amounts of desulfo-glucosinolates were then converted to µmol g^{-1}
325 fresh tissue weight (FW).
326 Using GLMs, we first tested whether total glucosinolate concentrations were
327 significantly induced following the extended period of larval herbivory across all
328 populations, then tested for the significance of induction within the low-, intermediate- and
329 high-elevation classes. Next, we tested whether individual glucosinolates showed
330 significant induction, using individual GLMs and a false discovery rate (FDR) of 10% to
331 control for effects of multiple testing.
332
333 ***Variation in constitutive glucosinolate concentrations with increasing elevation***
334 Given the observed decline in herbivore damage with increasing elevation in the
335 field, we also tested whether constitutive chemical defences declined with increasing
336 elevation. We germinated seeds from 5 families for each of 16 populations (6 low, 5

intermediate and 5 high elevation). Seeds were stratified and then moved to a climate chamber (19ºC day, 14ºC night) for 7 days before thinning down to one seedling per cell. Leaf number and length of the longest leaf were recorded after 5.5 weeks. One fully expanded leaf per plant was weighed, flash frozen and freeze-dried for glucosinolate analysis, and the mass of the remaining aboveground fresh plant tissue measured as described above.

Glucosinolates were extracted, identified and quantified as described in the previous section. We tested for variation in total and individual glucosinolate concentrations with respect to a fixed effect of elevation (controlling for multiple testing using an FDR of 10%), then repeated the analysis using a fixed effect of population (and a FDR 10%). Due to differences between extraction sets in total glucosinolate amounts, extraction set was included as a fixed effect in all analyses. Furthermore, to evaluate the prediction that investment in defence declines with increasing growth rates, we tested for associations between total glucosinolate production and total aboveground mass, leaf number and maximum leaf length. Square root transformation of the response variable was used to improve model fit if inspection of model residuals suggested deviations from expectations under normality.

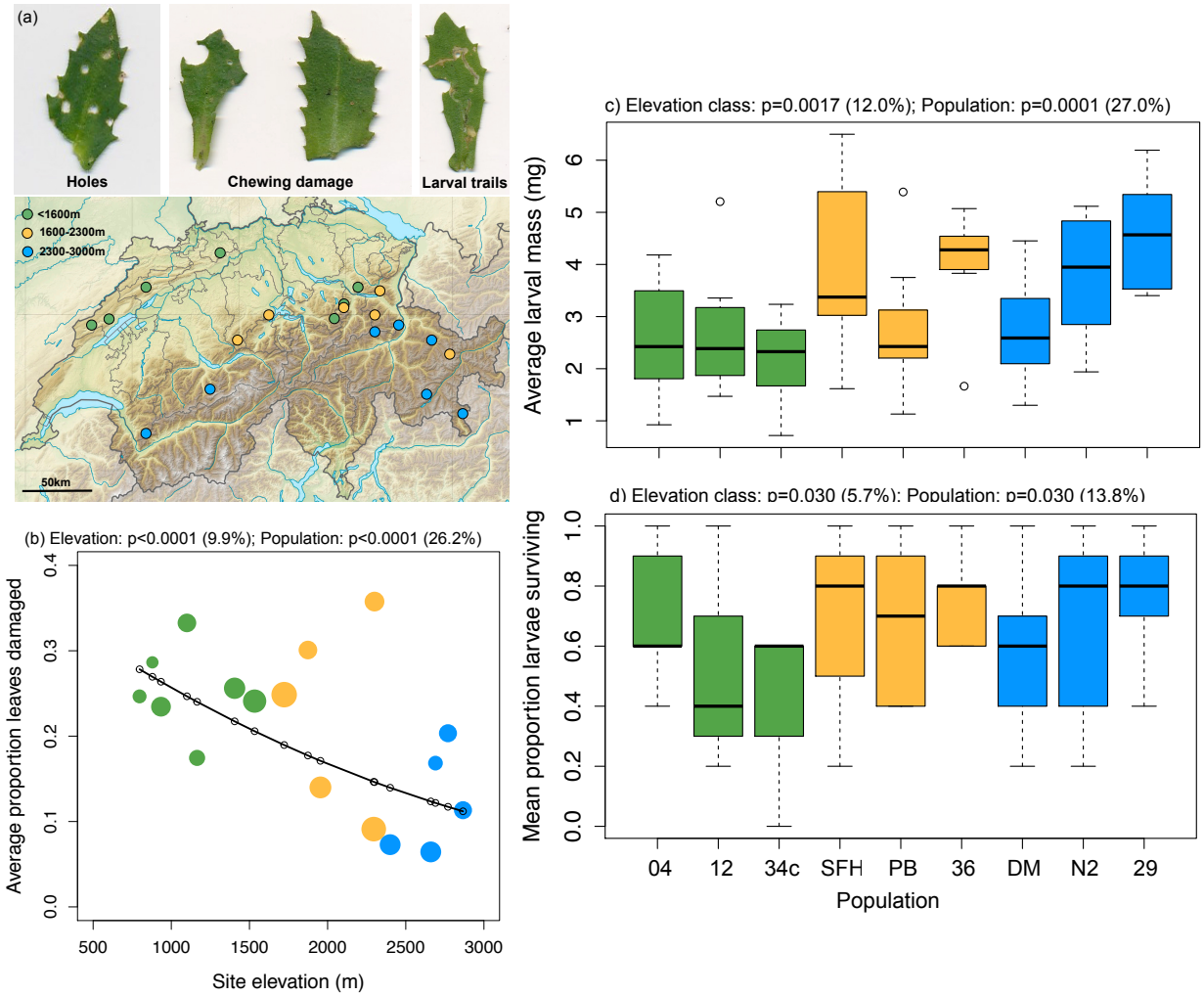
RESULTS

Leaf damage in the field declines with increasing elevation

Across the 16 field populations surveyed, we observed a decline in the proportion of leaves damaged with increasing elevation (Figure 1b), as well as in the proportion of plants showing different types of herbivore damage ($p<0.001$ for leaf holes, chewed edges and larval trails; Figure S1a-c). Molluscs and several specialist herbivores of Brassicaceae were observed feeding on *A. alpina* (see Figure S2). One damage type—pale leaf spots, which were difficult to attribute to a particular herbivore—displayed a significant increase with

363 increasing elevation (Figure S1d). Population-level effects explained 2.7x more variance in
364 the proportion leaves damaged than did elevation alone (population = 26.2%; elevation =
365 9.9%). Similarly, population explained 2.7-4.6x more variation in each damage type than
366 elevation (Figure S1), suggesting that population-specific genetic and environmental
367 influences account for the majority of variation in these traits. The average proportion of
368 damaged leaves per population increased with long-term average yearly temperature (R^2
369 =0.229; $F_{1,15} = 5.739$, p=0.03), although temperature was correlated with elevation and both
370 factors explained a similar amount of variation (elevation: $R^2 = 0.25$, temperature: $R^2 =$
371 0.23). This suggests that temperature might be important for explaining the elevational
372 gradient in herbivory.

Figure 1: (a) Photos of the three main types of herbivore-driven damage observed in populations and location of 19 study populations across Switzerland and their classification in to one of three elevation categories; (b) Decline in the average proportion of leaves damaged per population, with points weighted by sample size, a line indicating model fitted values (GLM binomial error) and the significance (and % explained variance) for elevation and population in separate GLMs. (c) Variation in average larval mass per plant (based on five larvae per plant after eight days and (d) proportion larvae surviving on nine populations (three low, three intermediate and three high). In (c) and (d) each population was represented by 8 plants. The base map of Switzerland in (a) was produced by Wikimedia commons users Eric Gaba and NordNordWest.



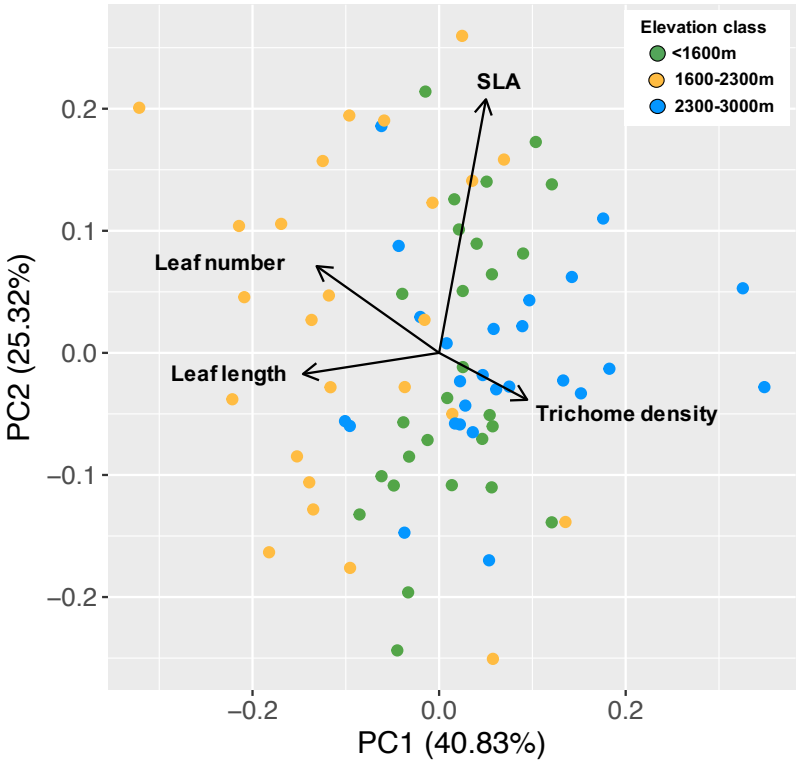
Populations from different elevations diverge in morphological and growth traits

Our field surveys showed that elevation had contrasting effects on plant growth form, and that this variation persisted under a common environment (Figure S3). In the field, plant leaf number varied significantly among populations, independent of changes in elevation, with population explaining 28.6% variance in the number of leaves ($F = 5.89$, $df = 16$, $p<0.0001$; Figure S3a). In particular, two intermediate populations, AalSFH and AalPB, produced particularly high numbers of leaves. By contrast, there was a decline in plant surface area with elevation ($F = 70.4$, $df = 1$, $p<0.0001$; Figure S3b), consistent with plants having smaller size at high elevations. Elevation and population explained a similar proportion of variance in plant size (elevation = 21.3% and population = 30.0%).

Experiments in which field-collected seeds from a subset of populations were grown in a common environment resulted in similar variation in plant growth form to that observed in the field. The number of leaves varied significantly among the nine populations ($F = 16.40$, $df = 8$, $p<0.0001$), but also between elevation classes ($F = 16.42$, $df = 2$, $p<0.0001$), with intermediate-elevation populations (particularly AalSFH and AalPB) showing significantly higher leaf production (Figure S4a). Maximum leaf length (a proxy for rosette size) was significantly reduced for populations from high elevations relative to both the low and intermediate elevation classes ($F = 25.54$, $df = 2$, $p<0.0001$; Figure S4b). For both leaf number and leaf length, the proportion of variance explained by population alone was greater than that explained by elevation class (by 1.9x and 1.3x respectively), highlighting the importance of population-level effects in shaping variation in these traits under common growing conditions. In a separate experiment using all 17 populations, aboveground dry mass (at 1 month of age) did not decline with increasing elevation ($F = 2.03$, $df = 1$, $p = 0.157$) or show differences among populations ($F = 0.85$, df -= 15,

421 p = 0.619; Figure S3c); however, maximum leaf length clearly declined with
422 increasing elevation (R^2 = 0.36, F = 60.52, df = 1, p<0.0001; Figure S3d).
423 When grown in a common environment, SLA (a proxy for leaf density) did
424 not vary among populations or elevation classes (Figure S4c), although a high
425 proportion of variance in SLA was associated with individual plants (genotypes)
426 (population alone: R^2 = 0.05, with random effect of family: R^2 = 0.80). Trichome
427 density varied significantly across populations (F = 16.3, df = 8, p<0.0001), but not
428 among elevation classes (X^2 = 5.839, df = 2, p = 0.054; Figure S4d). Both the highest
429 mean trichome density (population AalDM = 448.4 trichomes per cm^2) and the lowest
430 mean density (Aal29 =164.1 trichomes per cm^2; Figure S4d) were observed in high-
431 elevation populations.
432 Principal components analysis revealed evidence for genetic divergence
433 among low-, intermediate- and high-elevation populations along a growth-
434 morphology spectrum (Figure 2). Principal component loadings for different traits
435 showed that relative to high-elevation populations plants from intermediate-elevation
436 populations had larger rosettes, lower trichome densities and higher rates of leaf
437 production. Conversely, plants from high-elevation populations had smaller rosettes,
438 variable trichome densities and lower rates of leaf production. Finally, low-elevation
439 populations exhibited higher trichome densities and lower rates of leaf production
440 than intermediate-elevation plants, yet larger rosettes than plants from high-elevation
441 populations (Figure S4).
442
443 **Figure 2.** PCA summarising growth and morphological trait variation among plants
444 from the different elevation classes. The PCA is based on data on the number of
445 leaves produced, length of the longest leaf, specific leaf area (cm mg^{-1}) and number of
446 trichomes on adaxial (lower) surface from the same set of individuals. The arrows

represent the coefficients of the four variables (traits) on the two principal components (PC1 and PC2), so point in the direction where values of that trait are maximised. Points are coloured by elevation class.



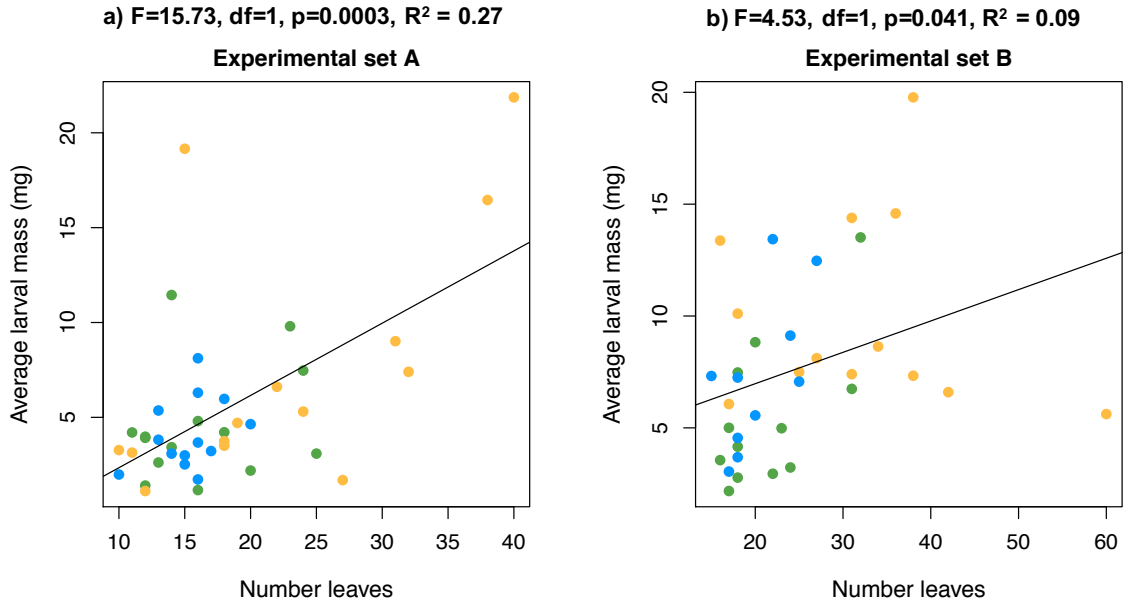
Herbivore performance and survival is reduced on plants from low-elevation populations

Herbivore performance assays with the specialist *Pieris brassicae*, conducted on nine populations, revealed a significant effect of elevation ($R^2 = 0.12$, $X^2 = 12.77$, df = 2, $p = 0.002$), with larvae showing significantly higher mass after 8 days feeding on plants from intermediate- and high-elevation populations than those feeding on plants from low-elevation populations (Figure 1c). Nevertheless, the proportion of variance in larval mass explained by population effects was 2.25x higher than that explained by elevation ($R^2 = 0.270$; $X^2 = 31.23$, df = 8, $p<0.001$). In particular, caterpillars feeding on the high-elevation populations Aal29 and AalDM exhibited very different mean (+/- S.E) larval masses (Aal29 = 4.56 +/-0.37mg and AalDM =

2.73 +/-0.37mg; Figure 1c). After 8 days on the plants, 64.2% of the larvae had survived. Survival was significantly lower on low-elevation plants (Binomial GLM: $X^2 = 7.00$, df = 2, $p = 0.03$); however, the amount of variance explained by elevation was low (5.7%; Figure 1d). Two low-elevation populations had the lowest larval survival rates (Aal34c = 45% larvae, Aal12 = 50%), while one intermediate- and one high-elevation population showed the highest rates of survival (Aal36= 75% and Aal29 = 78%; Figure 1d). These results suggest that high-elevation plants were generally more favourable hosts than low-elevation plants, despite clear population-level differences within elevation classes.

When elevation was replaced by plant growth and morphological traits in the model, we found that only total number of leaves had a significant positive effect on variation in larval performance ($R^2 = 0.23$; F = 24.2, df = 1, $p<0.0001$). This effect was partly due to the second experimental set of plants showing, on average, both more leaves and heavier larvae (due to space limitations, this set was assayed one week later than the first experimental set). However, separating the samples by experimental set confirmed a positive effect of number of leaves on larval mass in both groups (Figure 3; set A: F = 15.7, df = 1, $p<0.001$, $R^2 = 0.27$; set B: F = 5.70, df = 1, $p = 0.023$, $R^2 = 0.12$). Larvae did not eat all the tissue presented to them: on average only 18% of leaves had more than a quarter of leaf area removed for set A (maximum = 82% of leaves) or 12% for set B (maximum = 33% of leaves). However, there was variation among plants from different populations in the proportion of leaves showing any signs of damage (Figure S5a), and low-elevation populations showed a significantly lower proportion of leaves with >25% leaf area removed (Figure S5b). Together, these data suggest increased leaf production is associated with increased leaf quality for specialist herbivores.

Figure 3: Regression of variation in larval performance (average mass of three larvae after six days feeding on one plant) on variation in the number of leaves per plant for (a) experimental set A and (b) experimental set B. Relevant statistics for the effect of leaf number on variance in larval mass, including the amount of variance (R^2) explained by number of leaves, are reported above each graph. Colours represent different elevation classes (see key in Figure 2).

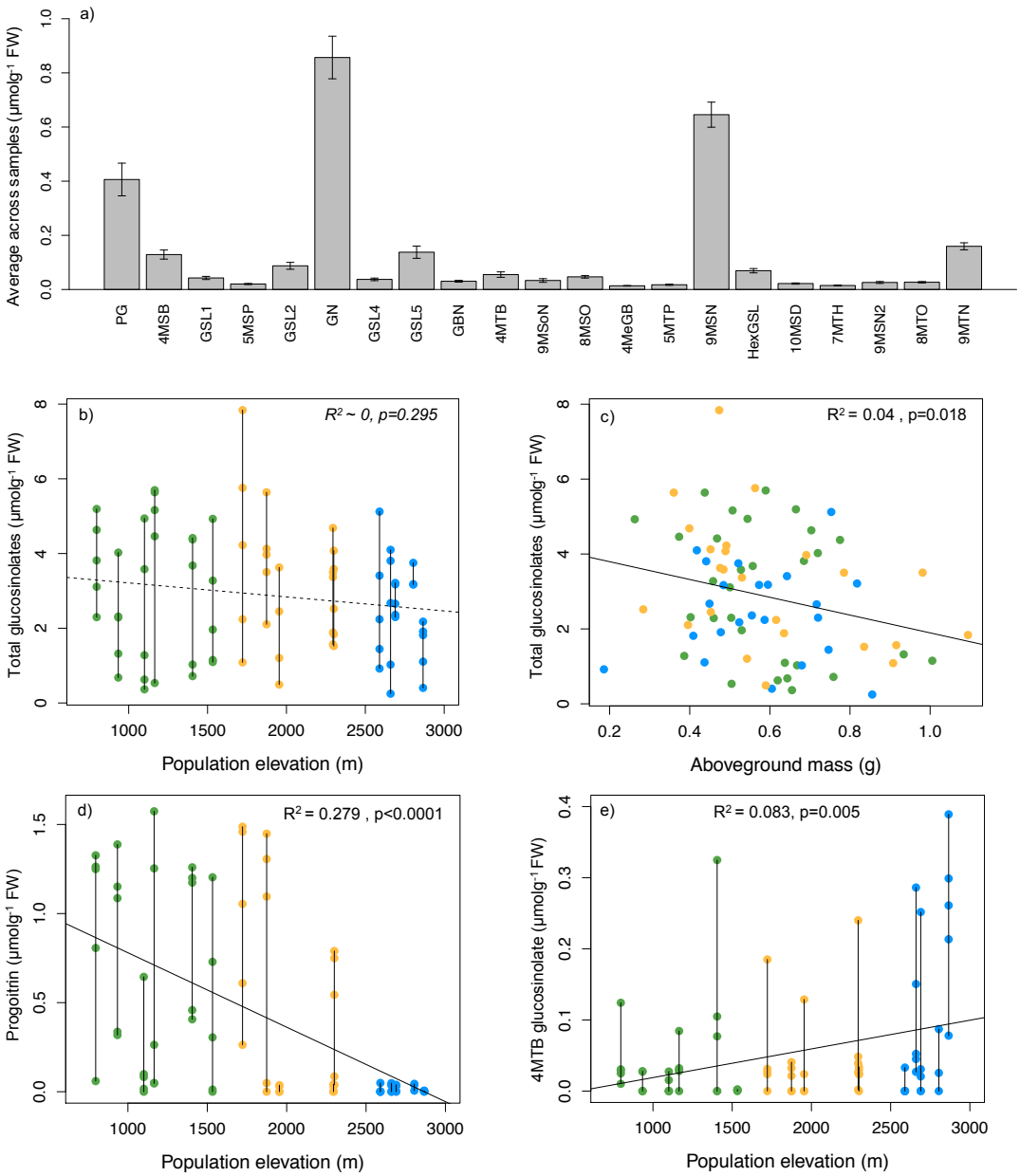


Several glucosinolate compounds exhibit significant trends with elevation

We identified 21 glucosinolates across all populations (Figure 4a, full details in Table S3), three of which (gluconapin, progoitrin and glucoarabin) accounted for more than 70% of total glucosinolate production (Figure 4a). Total constitutive glucosinolate levels showed no trend with increasing elevation ($F = 1.11$, $df = 1$, $p = 0.295$; Figure 4b), despite a more than 2.9-fold difference in mean total glucosinolate production across populations (ranging from 1.48µmolg^{-1} FW for the high-elevation population Aal29 to 4.30µmolg^{-1} FW for the intermediate-elevation population AalSFH; average across individuals: 2.88µmolg^{-1} FW). Total glucosinolate levels

were weakly negatively correlated with aboveground biomass ($R^2 = 0.038$; $F = 5.90$, $df = 1$, $p = 0.018$; Figure 4c), and length of the longest leaf ($R^2 = 0.03$; $F = 4.32$, $df = 1$, $p = 0.041$). Leaf number at the time of sampling was not significantly associated with total glucosinolates ($F = 1.78$, $df = 1$, $p = 0.186$), suggesting no connection between variation in rates of leaf production and investment in constitutive defences.

Figure 4: Variation in glucosinolate production across populations of *A. alpina.* (a) Average amounts across constitutive samples of individual glucosinolates ordered by increasing retention time (in micromoles per gram of fresh tissue, μmol g^{-1} FW, +/- one S.E.); Regression of variation in: (b) total glucosinolates on elevation; (c) total glucosinolates on aboveground mass; (d) levels of progoitrin on elevation; (e) levels of 4-(methylthio)butyl-glucosinolate on elevation. Regression lines are solid if relationship significant, and the adjusted R-squared and p-value are given. Vertical black lines connect samples from the same population in plots b, d and e, and different coloured points represent samples from low, intermediate and high elevation classes. Shorthand codes for glucosinolates are given in Table S3.



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524 Because total glucosinolate amounts can obscure biologically relevant

525 variation in individual compounds (Poelman, Galiart et al. 2008), we also tested

526 whether individual glucosinolates varied with elevation. This analysis revealed

527 significant trends with elevation for eight of the 21 compounds, with six declining

528 with increasing elevation and two increasing (10% FDR; Table S4). Progoitrin (PG)

529 showed the strongest decline with elevation ($R^2 = 0.28$), being consistently low in

530 high-elevation populations (Figure 4d), whereas 4-(methylthio)butyl glucosinolate

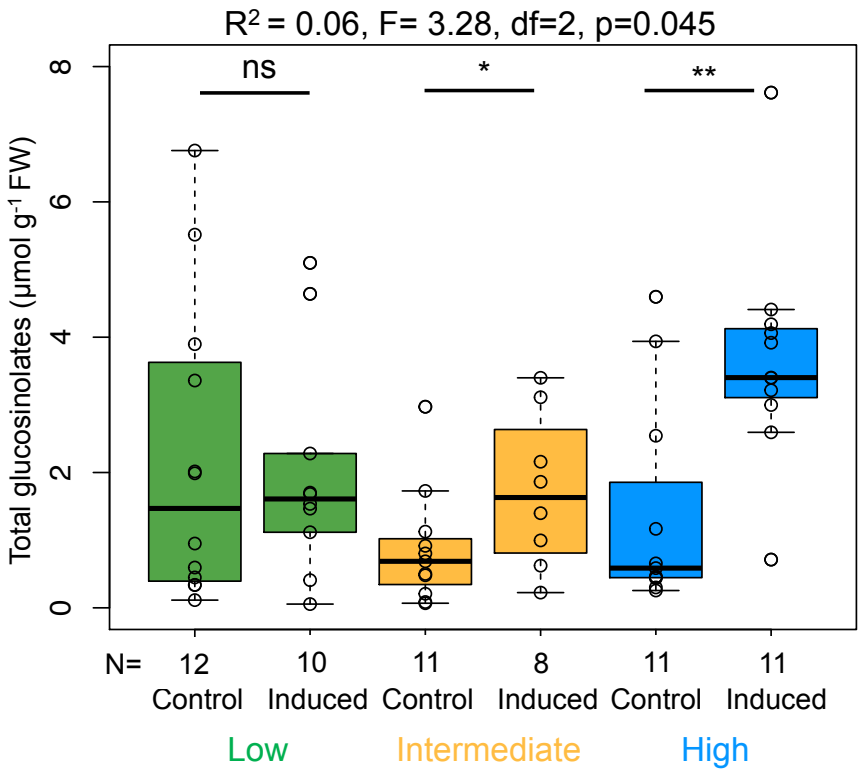
(4MTB) showed the strongest positive association ($R^2 = 0.08$; Figure 4e). Despite screening just five individuals per population, we also observed significant population-level variation for 13 individual glucosinolates 10% FDR; Table S5), with a small number of populations driving these effects. For example, one low-elevation population (Aal04) showed higher levels of 4-(methylsulfinyl)butyl glucosinolate (4MSB) relative to other populations, while another low-elevation population (AalCdV) showed elevated levels of three different glucosinolates (5-(methylthio)pentyl, 10-(methylthio)decyl, and glucobrassicanapin). Additionally, an intermediate-elevation population (AalSFH) showed elevated levels of two unidentified glucosinolates (GSL2 and GSL5), and one high-elevation population (Aal29) also showed an elevated frequency of 4-(methylthio)butyl glucosinolate (4MTB). These results thus reveal clear effects of elevation and population on individual glucosinolates, despite no such trends being observed for amounts of total glucosinolates.

Glucosinolate induction is stronger in high- and intermediate-elevation populations than in low-elevation populations

Total glucosinolates were significantly induced following feeding (for six days) by *Pieris* larvae (mean constitutive = 1.51 μmol g^{-1} FW; mean induced = 2.6 μmol g^{-1} FW*;* $p = 0.004$, $R^2 = 0.11$), with 12 of the 18 individual glucosinolates detected in this experiment showing significant induction (10% FDR; Table S6). When populations were grouped by elevation class, high- and intermediate-elevation populations showed significantly stronger total glucosinolate induction than low-elevation populations (Figure 5), although the variance explained by this interaction was low ($F = 3.28$, $df = 2$, $p = 0.045$; $R^2 = 0.06$). At the individual glucosinolate level, four of 18 glucosinolates showed significant elevation-by-induction interactions (10%

FDR; Table S6). On closer inspection, however, some of these differences were population-specific (e.g. strong induction in population Aal29 for 4MTB; Figure S6a). Interestingly, the unidentified glucosinolate GSL3 showed clear induction in both high-elevation populations (AalDM and Aal29; Figure S6b). By contrast, progoitrin (PG) showed no significant induction in any population (Figure S6c; Table S6) despite constitutive levels clearly declining in high-elevation populations (Figure 4d). Of the four individual glucosinolates that showed significant elevation-by-induction interactions, the two low-elevation populations consistently showed no effects of induction (e.g. Figure S6a,b). Taken together, these data support the hypothesis that chemical defence inducibility is stronger in higher elevation populations.

Figure 5: Change in total glucosinolates (in micromoles per gram of fresh tissue, μmol g^{-1} FW,) following herbivory across low-, intermediate- and high-elevation classes, with individual data points given on the boxplots. Each elevation class consists of data from two populations. Control treatments and herbivory-induced treatments are marked, with the significance of the induction effect for each elevation class given, as analysed with separate linear models (ns = $p>0.05$, * = $p<0.05$, ** = $p<0.001$).



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580 **DISCUSSION**

581 Our results provide evidence for considerable genetic divergence in multiple
582 growth and defence traits within an alpine plant species across its elevational range.
583 Indeed, the patterns of trait variation we observed among *Arabis alpina* populations
584 are comparable to divergent growth-defence "syndromes" previously described for
585 species that occur at different elevations (Defossez, Pellissier et al. 2018, Kergunteuil,
586 Descombes et al. 2018). This suggests that *A. alpina* can adapt to environmental
587 conditions that vary with altitude; however, our findings also reveal extensive
588 population-level variation in many growth and defence traits that is independent of
589 elevation. In particular, our results reveal genetic divergence among high-elevation
590 populations in traits associated with herbivore resistance and tolerance, suggesting

591 that this alpine plant might be able to adapt to predicted increases in herbivore
592 pressure at high elevations due to climate change.
593 Consistent with the findings of many previous studies (e.g. Garibaldi,
594 Kitzberger et al. 2011, Pellissier, Roger et al. 2014, Rokaya, Dostálek et al. 2016,
595 Moreira, Petry et al. 2018), we observed reduced rates of herbivore damage at high
596 elevations. This pattern could be explained by reduced herbivore pressure at these
597 elevations—which might favour corresponding reductions in defence investment—
598 but could also arise if high-elevation plants suffer less herbivory because they are
599 better defended (Rasmann, Pellissier et al. 2014). Larval performance assays in a
600 common (climate-chamber) environment indicated that our intermediate- and high-
601 elevation populations were generally more palatable for herbivores than low-elevation
602 populations, consistent with reduced defence investment in high-elevation
603 populations. We did not observe a decline in total glucosinolate levels with increasing
604 elevation, but did find significant elevational trends in several individual
605 glucosinolate compounds, six of which exhibited significant declines with increasing
606 elevation, while two exhibited significant increases. This pattern is generally
607 consistent with an overall reduction in glucosinolate defences at high elevation,
608 although additional experiments exploring how variation in these individual
609 glucosinolates affects the performance of specialist and generalist herbivores would
610 be necessary to confirm this.
611 When the risk of herbivory is unpredictable—as is often the case at high
612 elevations (Descombes, Marchon et al. 2017)—and the costs of continuously
613 producing constitutive defences are high (e.g. Zangerl and Rutledge 1996), selection
614 may favour investment in defences that are inducible upon herbivore attack (Moreira,
615 Mooney et al. 2014, Pellissier, Roger et al. 2014, Defossez, Pellissier et al. 2018,
616 Moreira, Petry et al. 2018). Alternatively, the limited resources available at high

617 elevations may favour greater investment in constitutive rather than induced defences
618 to defend leaves that are costly to replace (Coley, Bryant et al. 1985, Moreira,
619 Mooney et al. 2014, Pellissier, Moreira et al. 2016). While total constitutive
620 glucosinolates did not decline with increasing elevation in our study, high-elevation
621 *A.alpina* populations did show the strongest induction of total glucosinolates
622 following herbivory. We also observed significant induction of many individual
623 glucosinolates, yet found little evidence that the significant elevational trends
624 observed for constitutive levels of individual glucosinolates were associated with
625 differences in the strength of their inducibility among populations. Our observation of
626 increased inducibility at high-elevations is consistent with findings from several
627 recent studies (Rasmann, Buri et al. 2014, Galman, Petry et al. 2018), but notably
628 differs from the pattern observed in the field among different *Cardamine* species (also
629 members of the Brassicaceae family), where low-elevation species showed lower
630 levels of constitutive glucosinolates and greater inducibility relative to high-elevation
631 species (Pellissier, Moreira et al. 2016). Our glucosinolate data hints at the absence of
632 a strong trade-off between constitutive and induced chemical defences in *A. alpina*;
633 however, definitively establishing a trade-off between constitutive and induced
634 defences would require measuring defence induction in a greater number of
635 populations than used in the present study.
636 In addition to the observed elevational trends in defence traits, populations at
637 similar elevations exhibited significant divergence in many of these traits. Previous
638 studies have also reported trait variation among populations independent of
639 elevational gradients (Rokaya, Dostálek et al. 2016, Pfennigwerth, Bailey et al. 2017).
640 However, our study design explicitly included replication at the population level
641 within elevation classes, allowing us to estimate the relative contributions of
642 population and elevation to trait variation. We found that population-level effects

643 explained 2.25x more variation in herbivore performance than elevation alone.
644 Similarly, while total glucosinolate levels did not vary significantly among
645 populations, variation in many individual glucosinolates was better explained by
646 population-level effects than by elevation. Differences in local herbivore communities
647 have previously been linked to among-population variation in glucosinolate defences
648 over short geographic distances (Gols, Wagenaar et al. 2008, Newton, Bullock et al.
649 2009), and in our study population effects explained 2.6x more variation in field leaf
650 herbivore damage than elevation alone, suggesting that local variation in herbivore
651 pressure, independent of elevation, might drive some of the observed variation in
652 defence traits. However, to explicitly link population-level variation in defence traits
653 with geographic variation in herbivore pressure it would be necessary to characterise
654 herbivore communities and measure climatic variables at finer spatial and temporal
655 scales than was possible in our study. We should also note that because our assays
656 employed seeds collected directly from the field, we cannot exclude the possibility
657 that maternal effects also contribute to the observed population-level variation.
658 To better understand *A. alpina* adaptation to varying herbivore pressures
659 across elevations, we also documented elevational trends in multiple traits associated
660 with growth and morphology that might directly or indirectly affect plant interactions
661 with invertebrate herbivores (Coley, Bryant et al. 1985, Herms and Mattson 1992). A
662 principal components analysis combining data for two growth traits, trichome density
663 and SLA, revealed syndromes associated with different elevations: low-elevation
664 populations were characterised by high trichome densities, large rosette sizes, and low
665 rates of leaf production compared to populations from other elevations; meanwhile,
666 high-elevation populations had smaller rosettes than low-elevation populations and
667 lower rates of leaf production than intermediate-elevation populations, but highly
668 variable trichome densities; and intermediate-elevation populations were

669 characterised by generally low trichome densities, but larger rosette sizes and higher
670 rates of leaf production than populations from other elevations. As our measurements
671 were based on plants grown from seeds in a common environment, these results
672 indicate a significant genetic contribution to these phenotypic syndromes. These
673 patterns of trait divergence in *A. alpina* are broadly consistent with the growth-
674 defence syndromes previously described for *Cardamine* species from different
675 elevations (Defossez, Pellissier et al. 2018), where smaller size was associated with
676 high-elevation species, and increased biomass production with low- and intermediate-
677 elevation species. However, other aspects of these syndromes, including changes in
678 leaf density and in constitutive chemical defences, were more pronounced in that
679 system than in the current study, perhaps reflecting greater divergence in functional
680 traits among vs within species, or the fact that their study sampled traits only under
681 field conditions, while ours measured traits in a common environment.
682 Under both field and growth-chamber conditions, we found that *A. alpina*
683 plants from the highest elevations produced smaller rosettes with fewer leaves than
684 plants from lower elevations, consistent with evidence from a previous common-
685 garden experiment conducted in the field with French populations of *Arabis alpina*
686 (de Villemereuil, Mouterde et al. 2018). Growth rates have also been linked to
687 survival and reproductive effort in field populations of *A. alpina (*Andrello, de
688 Villemereuil *et al.* 2016*),* so together these lines of evidence suggest an adaptive role
689 of these growth traits in reducing exposure to local abiotic conditions at high
690 elevations (Körner, Neumayer et al. 1989, Byars, Papst et al. 2007, Körner 2007,
691 Read, Moorhead et al. 2014). By contrast, our observation of larger rosette sizes in
692 low- and intermediate-elevation populations could reflect an adaptive response to
693 increased competition from other plants under better growing conditions (see photos
694 comparing low and high-elevation habitats in Figure S7). In contrast to low- and high-

695 elevation populations, intermediate-elevation populations showed generally higher
696 rates of leaf production under both field and common-garden conditions, suggesting a
697 genetic basis for this trait. Based on similar observations of growth form variation
698 across species at different elevations, Defossez *et al.* (2018) hypothesized that high
699 rates of herbivory at intermediate-elevations may select for elevated leaf production
700 as a form of herbivore-tolerance.

701 The hypothesis that herbivore tolerance is favoured at intermediate-elevations
702 also fits with our trichome data. We observed significant variation in trichome density
703 among populations that was largely independent of elevation. However, plants from
704 low-elevation populations were characterised by consistently high trichome densities,
705 which could represent an adaptive response to an elevated frequency of encounters
706 with herbivores (e.g. Løe, Toräng et al. 2007) or a response to abiotic factors such as
707 increasing aridity (e.g. Kessler, Siorak et al. 2007). Meanwhile, intermediate-
708 elevation populations are also exposed to high rates of herbivory in the field, but
709 showed generally low trichome densities. This low investment in physical defence
710 combined with elevated levels of leaf production observed in these populations, is
711 consistent with a strategy of herbivore tolerance.

712 It is notable that many growth and morphological traits exhibited significant
713 variation across populations even within the three elevation classes (low, intermediate
714 and high). In particular, trichome density significantly varied among high-elevation
715 populations, with population AalDM showing much higher trichome densities relative
716 to the other populations (AalN2 and Aal29). Population-level variation in this putative
717 defensive trait may partly explain the reduced herbivore performance on plants from
718 AalDM relative to Aal29, where mean larval mass was 1.7x higher for larvae feeding
719 on Aal29 than AalDM. Such genetic divergence in plant defences among populations
720 at high elevations would not have been observed if populations from different

721 elevations were pooled for experimental testing, as has been done in some studies
722 (e.g. Ereli, Ayres et al. 1998, Pellissier, Roger et al. 2014, Rasmann, Buri et al. 2014).
723 These results suggest that high-elevation populations may not be consistently
724 vulnerable to the predicted changes in herbivore pressure with ongoing climate
725 change.

726 As discussed, selection by abiotic and biotic factors may be responsible for
727 population-level variation in anti-herbivore defence investment across the elevational
728 range of this species. However, another potential explanation for the observed
729 population-level effects in defence and growth/morphological traits is that *A. alpina*
730 populations sampled from different areas of the Alps may derive from distinct genetic
731 lineages. After the last glaciation the Alps were colonised by *A. alpina* from multiple
732 glacial refugia around the Mediterranean (Koch, Kiefer et al. 2006, Rogivue, Graf et
733 al. 2017), and it is unknown to what extent these distinct postglacial histories (and
734 associated genetic drift) might have influenced the current composition of traits in this
735 species. An interesting next step will therefore be to identify patterns of neutral
736 genetic structure across our *A. alpina* samples to determine the extent to which
737 divergence in defence, growth and morphological traits are reflected in patterns of
738 neutral genetic structure.

739

740 **CONCLUSIONS**

741 This study documents genetic variation in multiple growth and defence-related
742 traits that is likely important for adapting to spatially varying biotic conditions across
743 the elevational range of an alpine plant. Importantly, while many traits showed
744 significant elevational trends, population-level effects consistently explained more
745 trait variation than elevation. Although, the precise selective forces driving these
746 differences remain uncertain, the presence of genetic variation in growth and defence

traits across the range of this alpine species may facilitate evolutionary responses of this species to changes in biotic interactions associated with climate warming. Indeed, recent theoretical and empirical work suggests that local adaptation can have implications for the response of species to rapid environmental change (Pelini, Keppel et al. 2010, Valladares, Matesanz et al. 2014), and understanding the extent of intraspecific variation in key traits is predicted to be important for accurately forecasting the response of individual species to such changes (Urban, Bocedi et al. 2016). In particular, our assessment of variation both within and across elevation classes suggests that high-elevation populations of *A. alpina* are not consistently more vulnerable to herbivores than intermediate- and low-elevation populations. Future work should test whether population-level genetic variation in similar sets of traits exists within species with more restricted elevational distributions, as such species are predicted to be particularly vulnerable to ongoing environmental change.

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AUTHOR'S CONTRIBUTIONS

JB, AW, MCM and CMDM conceived the ideas, designed methodology and wrote the manuscript; JB collected and analysed the data; All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data has been deposited in the Dryad repository:
http://datadryad.org/resource/doi:10.5061/dryad.ff11k13

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