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Differences in herbivore damage and performance among
Arctium minus (burdock) genotypes sampled from
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3 **(burdock) genotypes sampled from a geographic gradient: a common garden**
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Abstract

Performance of plant species does not necessarily decline as they approach their geographic range limits. One reason for this may be a loss of natural enemies in marginal populations. Such patterns have been found in native species, but also may occur for exotics if they have not already escaped their herbivores in invaded regions. For instance, the Eurasian biennial *Arctium minus* (common burdock) is attacked by a variety of native and introduced insects in its new North American range. Previously, research has shown that damage by these herbivores strongly decreases towards the northern range limit of this species. This gradient might reflect a genetic cline in resistance to herbivores, or geographic variation in herbivore abundance. To distinguish between these possibilities, herbivore damage to leaves and seeds of *A. minus* was measured in a common garden experiment with genotypes sampled from 11 populations along a 550 km transect extending from southern Ontario towards burdock's northern range limit. As well, a freezing tolerance experiment was performed with the important lepidopteran seed predator *Metzneria lappella*, and palatability experiments were performed with two generalists, the snail *Cepaea nemoralis* and the moth *Trichoplusia ni*. Although there were some differences in damage among populations, results indicated that latitudinal differences in herbivore damage are not explained by genotypic differences among populations, but instead are likely to result from the absence of herbivores from colder sites. Escape of *A. minus* from its usual herbivores may increase performance of marginal populations, and contribute to future spread.

Key words: *Arctium minus*; burdock; common garden; herbivory; latitudinal gradients; *Metzneria lappella*

Introduction

Geographic range limits of plant species can result from gradients in physical conditions, such as temperature or water availability; consequently, as a species approaches its range limit, population size and fitness may decrease reflecting an increasingly unsuitable environment (Geber 2008; Kawecki 2008; Sexton et al. 2009). For instance, Jump and Woodward (2003) found that seed production and population density of *Cirsium acaule* and *C. heterophyllum* declined approaching the northern range limits of these thistles. Similarly, a study of purple loosestrife (*Lythrum salicaria*) showed that plants were smaller and less fecund approaching its range limit (Colautti et al. 2010). A review by Sexton et al. (2009) documented numerous such cases; for example, 28 of 39 studies that transplanted organisms beyond their range margins reported a decline in fitness. Similarly, Hargreaves et al. (2014) found performance declined in 75% of cases of organisms transplanted beyond their range limits. Still, while such patterns are common, they are not universal. Instead, there are numerous cases in which populations at range margins perform as well or better than central populations (Kawecki 2008; Sexton et al. 2009; Hargreaves et al. 2014); for instance, Garcia et al. (2010) showed that reproduction and population growth rates of marginal populations of a lady slipper orchid (*Cypripedium calceolus*) were as good as or better than the performance of central populations.

Such maintenance of performance close to range margins might reflect geographic trends in interactions with natural enemies, if damage is reduced in marginal sites. As one example, Alexander et al. (2007) found that populations of eastern woodland sedge (*Carex blanda*) at its range margin were free from specialized diseases and seed predators, and had correspondingly greater individual size and seed production. Other studies have found that herbivory on a given plant species often declines with increasing latitude (e.g., Pennings et al. 2007, 2009; Kozlov 2007; Vaupel and Matthies 2012), though the generality of this trend remains controversial: a meta-analysis by Moles et al. (2011) reported that only 37% of studies found higher herbivory at lower latitudes. A decoupling of plants and their

herbivores could occur if herbivores are scarce in marginal sites, either because they are unable to tolerate local conditions, or because they are unable to reach or demographically persist in these locations. For instance, distributions of insect herbivores are sensitive to variation in climate (Hill et al. 2011): if plants are more cold-tolerant than their herbivores, these enemies might be excluded from host populations at colder latitudes or altitudes; alternatively, herbivores may be prevented from reaching otherwise suitable sites by geographical barriers, or because of difficulties in locating and persisting in small or isolated marginal populations (e.g., Vaupel and Matthies 2012). As well, feeding activity of those insects which do occur in more northern sites may be reduced by colder temperatures. Finally, locally-adapted plants in marginal sites simply might be more resistant to herbivores. This might occur if slower growth near range margins selects for greater investment into anti-herbivore defences, as suggested by the Resource Allocation Hypothesis (Coley et al. 1985). Moles et al. (2011) used a meta-analysis to provide evidence that defences in plants increase at higher latitudes, and suggest that this may be explained by the higher cost of damage in lower-productivity sites.

Most studies of geographic gradients in herbivory have focussed on native species (e.g., Pennings et al. 2007, 2009; Kozlov 2007; Vaupel and Matthies 2012; Woods et al. 2012; Lakeman-Fraser and Ewers 2013); however, similar considerations may influence the distribution and performance of non-natives as well. The Enemy Release Hypothesis (ERH) argues that invasive species have an advantage over natives because they have lost specialized herbivores during migration to new regions (Keane and Crawley 2002; Torchin and Mitchell 2004; Mitchell et al. 2006). However, invasive plants rarely escape all enemies (e.g., Hill and Kotanen 2012); instead, most are subject to damage by generalists, and in some cases by specialists adapted to close relatives, co-introduced with the invader, or deliberately introduced for biocontrol. As an invader spreads within its new range, these enemies may or may not spread with it, depending on their dispersal ability and/or ability to tolerate the environments of newly colonised areas. For example, plants expanding their range in response to

climate change potentially may escape their usual natural enemies (Gilman et al. 2010; Morriën et al. 2010; Van der Putten et al. 2010).

Kambo and Kotanen (2014) studied European common burdock (*Arctium minus*) in its invaded North American range, documenting a striking latitudinal gradient in herbivore damage. Folivory and pre-dispersal seed predation both decreased sharply between temperate southern Ontario and the boreal range edge of this species; in contrast, production of viable seeds strongly increased, while there were small increases in other measures of performance. However, the causes of this gradient remain unclear. Here, we report results of a study designed to determine whether this gradient in herbivore damage reflects an intrinsic gradient in traits of the plants themselves, or a gradient in exposure to herbivores. Our principal question was whether the gradient in damage observed in the field would be preserved when genotypes sampled from a latitudinal transect were grown together in a common garden in a high-damage environment. We also conducted accessory palatability and freezing-tolerance experiments to further investigate possible causes of this gradient. Our results provide evidence that the documented differences in herbivory among *Arctium* populations do not reflect a genotypic cline in herbivore resistance; instead, they likely reflect a decline in populations and/or activity of herbivores, with potential implications for the further spread of this invader.

Methods

Study Species

Common burdock (*Arctium minus* (Hill) Bernhardt: Asteraceae; hereafter "burdock") is an invasive Eurasian biennial that now occurs from the southern United States to every province of Canada (Gross et al. 1980). Burdock is self-compatible (Gross et al. 1980), producing capitulae (seedheads) that each contain 10-75 seeds (Kambo and Kotanen 2014). The outer layer of each capitulum is covered in hooks which allow it to adhere to animals and disperse its seeds (Gross et al. 1980). In its first year following germination, burdock grows to approximately 35-50cm in height as a

rosette; after overwintering, it continues growth, ultimately producing inflorescences from 50-150cm in height (Kambo and Kotanen 2014).

Burdock in Ontario is attacked by wide variety of herbivores (Kambo and Kotanen 2014).

Common leaf miners include the North American agromyzid flies *Liriomyza arctii* Spencer (serpentine miner) and *Calycomyza flavinotum* Frick (blotch miner). Chewing folivores include a wide range of taxa such as Keeler's spurthroated grasshoppers (*Melanoplus keeleri* (Thomas)), grove snails (*Cepaea* cf. *nemoralis* (Linnaeus)), and a variety of Lepidoptera. Finally, burdock experiences high rates of attack by pre-dispersal seed predators, primarily the introduced burdock seedhead moth (*Metzneria lappella* (Linnaeus)), but also the tephritid flies *Tephritis bardanae* (Schrank) and *Cerajocera tussilaginis* (Fabricius); all lay eggs in the capitulum and larvae eat the seeds within.

Common Garden Experiment

In the summer of 2010, approximately 100 capitulae were collected from each of five burdock plants at 11 of the 12 Ontario locations surveyed by Kambo and Kotanen (2014) (excepting Moosonee). These locations represent a 550km latitudinal transect from Newmarket (44°02'N, 79°32'W) to Cochrane (49°04'N, 81°01'W); see Kambo and Kotanen (2014) for details. In May 2011, seeds from these capitulae were germinated and grown in a greenhouse until seedlings were approximately 10cm tall. A common garden experiment was then established during June 2011 at the Koffler Scientific Reserve near Newmarket (<http://ksr.utoronto.ca>). A 20m x 20m plot was plowed, and 20 seedlings from each of the 11 locations were then planted in a 1m regular grid (total N= 220). To reduce transplant shock, seedlings were watered for 2-3 days after planting. Plants were not further watered or fertilized, in order to avoid making them larger, more vigorous, or more palatable than wild individuals; this may have increased their level of stress, but was done to avoid unnaturally altering their attractiveness to herbivores. This plot was revisited in August 2011 to observe initial herbivore damage, then in May and August 2012 to observe lifetime survival and plant traits as well as herbivory.

In 2011, the initial percent of leaf area damaged by folivores was roughly estimated for each plant by superimposing a 0.6cm x 0.6cm grid on the surface of the largest available leaf (after its removal from the plant), and counting the squares occupied by holes. The area damaged was then divided by the total leaf area, estimated as a half-ellipse. In 2012, an improved procedure was used: damage was estimated for each plant by removing a basal leaf, the fifth, and tenth stem leaves and comparing damaged areas with models created by scanning leaves and digitally removing holes equal to 1%, 2%, and 5% of area. Total damage to each leaf was summed, then values for these three leaves were averaged before analysis, to give one % damage value per plant. A dissecting microscope was used to count trichomes along 0.5 cm of the central leaf vein on the underside of these three leaves, and leaf toughness was measured for them near their centres (but avoiding the central vein) using a 3mm Chatillon penetrometer. Due to an extremely hot summer in 2012 (Environment Canada: <http://www.weatheroffice.gc.ca>), leaves of many individuals withered before the final sampling (especially for northern populations), precluding measurements of herbivore damage and leaf traits. Sample size for those measurements therefore varied from 2-16 individuals per population.

Survival, height, stem circumference, and the total number of capitulae per individual also were measured. Total above-ground vegetative and reproductive (capitular) dry mass were determined by collecting plant material in paper bags, drying them at 50C in a drier over for at least 48 hours, and weighing them on a lab balance. Ten capitulae per flowering plant similarly were weighed and examined to determine average mass and the number of seeds and moth larvae they contained.

Palatability Experiment

Two generalist herbivores were used in lab palatability experiments: cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae), was purchased from the Canadian Forest Service (<http://insect.glf.c.forestry.ca>) and the snail *Cepaea cf. nemoralis* was collected locally. *T. ni* is a generalist herbivore considered to be a major pest in North America, feeding on agricultural crops such

as celery, lettuce, and spinach. *Cepaea nemoralis* is also a common generalist introduced to North America from Europe (Ozgo and Schilthuizen 2012).

Seeds from Newmarket, Cochrane, and an intermediate location (Huntsville: 45°20'N, 79°13'W) were germinated and maintained in a greenhouse in March 2012. Kambo and Kotanen (2014) found plants at these locations strongly differed in levels of herbivore damage; in particular, Newmarket populations exhibited some of the highest levels of folivory observed, while damage to Cochrane populations was very low. For the *T. ni* palatability experiment, a 5cm leaf disk was cut from each of two leaves sampled from 10 individuals per source location (N = 60 leaf disks = 2 disks x 10 plants x 3 populations). For the experiment with *C. nemoralis*, a single leaf disk was made from each of 20 individuals per location (N = 60 disks = 1 disk x 20 plants x 3 populations). The weight of each leaf disk was measured before it was presented to the herbivores.

Both caterpillars and snails were exposed for at least three days to *Arctium minus* leaves collected near the University of Toronto Mississauga campus, in order to familiarize them with this species; they then were then starved for one day. Herbivores were then weighed and randomly assigned to leaf disks: Petri dishes containing a leaf disk and a single *T. ni* or *C. nemoralis* were placed in a chamber with 90% humidity under a 14:10 hour 25C:10C light : dark cycle. Twenty leaf disks from each of the three locations also were placed in this chamber without herbivores as a control for water loss. *T. ni* and *C. nemoralis* were removed from leaf disks after three days and one week, respectively, reflecting their respective rates of tissue consumption. Leaf disks and herbivores were then weighed, and the percent of disk area damaged was measured by scanning and analysis with ImageJ software (<http://rsb.info.nih.gov/ij>). For the *T. ni* trial, data from the two leaf disks sampled from the same burdock individual were averaged prior to statistical analyses; this was not necessary for the *C. nemoralis* trial, since each plant provided only a single leaf disk. Replicates in which animals died, escaped, or refused to eat were excluded from analyses.

Freezing Tolerance Experiment

Twenty capitulae from each 20 individuals (N=400 capitulae) were collected near Newmarket during January 2012, after the arrival of cold winter temperatures. These were divided evenly into four different temperature treatment groups: -44C, -34C, -29C, and -24C. The warmer three temperatures were chosen based on the coldest temperature recorded respectively at Cochrane, Barrie, and Newmarket during January 2011 (Environment Canada 2012); the -44C treatment was added to represent January temperatures at sites north of the study area. These groups were then placed in freezers at their respective temperatures for two days. Capitulae were then opened and surviving and dead larvae of *Metzneria lappella* were counted.

Statistical Analysis

The influence of latitude of origin on common garden data (herbivory and plant traits) was analyzed with ANCOVAs using an REML approach, with latitude as a continuous variable and location of origin as a nominal random effect (Crawley 2007); differences among locations also were directly assessed using one-way ANOVAs. Survival of plants was examined using X^2 tests and linear regressions. Results of the palatability experiments (leaf area removal, leaf weight change, and herbivore weight change) were analyzed with one-way ANOVAs. Finally, X^2 analyses were performed to investigate differences in larval mortality among temperatures in the freezing tolerance experiment, while larval weights were compared with one-way ANOVAs. Averaged proportional data were arcsin-transformed before analysis ($y' = 2 * \arcsin(y^{.5})$), since the presence of zeroes made logit transformations problematic. ANOVA data were assessed for homogeneity of variance among populations (Levene's test); results of Welch's ANOVA for unequal variance are reported where data were inhomogenous. Substantial deviations of residuals from normality were rare (normal quantile plots), but are noted below. All analyses were performed with JMP 10 (SAS Institute Inc.).

Results

Common Garden Experiment

Survival

By May 2012, 57 of the 220 individuals originally planted had died. There were marginally significant differences in this first-year mortality among populations (Pearson $X^2_{10} = 18.138$; $p = 0.053$) but no obvious latitudinal trends. At minimum, 10 living individuals still were available for sampling per population in 2012. By the end of the experiment, all of these plants had either flowered (155) or still survived as rosettes (8 plants, of which half were from the Newmarket population). A regression on population means indicated that the proportion of plants dying before reproduction did not depend on latitude ($F_{1,9} = 0.482$, $p = 0.505$; Figure 1A). This analysis assumes non-flowering plants may flower in the future; however, deleting them from the analysis did not alter the results ($F_{1,9} = 0.667$, $p = 0.435$). Forty-five of the surviving 163 plants had largely withered before the final sampling. The probability of withering increased with latitude of origin ($F_{1,9} = 16.799$, $p = 0.003$; Figure 1B); excluding rosettes slightly strengthened this result ($F_{1,9} = 18.186$, $p = 0.002$). Because of their small number, uncertain status, and because they did not permit full sampling of plant traits, the 8 rosettes remaining in 2012 were excluded from all further analyses.

Folivory and seed predation

At the initial sampling in August 2011, five plants had already disappeared and presumably died, resulting in a sample size of 215. For these plants, the percentage of leaf area damaged was very low and did not vary with latitude ($F_{1,9,2} = 0.148$, $p = 0.709$) (Figure 2A) or among populations ($F_{10,204} = 0.904$, $p = 0.531$); transformed data still were inhomogeneous and non-normal, but a Welch's ANOVA produced similar results ($p = 0.601$). Only chewing damage was observed; leaf miners were absent. In 2012, herbivore damage could be reliably determined only for the 110 flowering plants which had not yet withered. Damage marginally differed among populations (ANOVA: $F_{10,99} = 1.782$, $p = 0.074$),

despite mild deviations from homogeneity ($p = 0.026$) and normality; when an ANOVA for unequal variances was used, significance improved (Welch's $p = 0.005$) (Figure 2B). Despite a hint of a declining trend, damage did not depend on latitude (ANCOVA: $F_{1,11.1} = 1.712$, $p = 0.217$) (Figure 2B). Leaf miners still were absent. Including withered but still identifiable leaves did not alter these results.

In 2012, capitulae were examined for all 155 plants that flowered, including withered individuals. Insect larvae, primarily *Metzneria lappella*, had attacked 46% of capitulae; nonetheless, the fraction of capitulae attacked did not depend on latitude (ANCOVA: $F_{1,6.1} = 1.422$; $p = 0.278$) or vary among populations (ANOVA: $F_{10,144} = 0.458$, $p = 0.914$) (Figure 2C). There was no significant difference in the number of larvae per capitulum in either ANCOVA ($F_{1,8.4} = 0.274$, $p = 0.615$) or ANOVA models ($F_{10,144} = 0.503$, $p = 0.886$) (Figure 2D).

Plant characteristics

In 2012, height and reproductive characteristics could be determined for all 155 flowering plants. There were no significant trends with latitude in ANCOVA analyses of above-ground vegetative biomass ($F_{1,7.8} = 0.809$, $p = 0.396$), total reproductive biomass ($F_{1,8.5} = 0.052$, $p = 0.826$), height ($F_{1,7.9} = 0.255$, $p = 0.628$), stem circumference ($F_{1,8.1} < 0.001$, $p = 0.984$), number of capitulae ($F_{1,8.5} = 1.227$, $p = 0.298$), number of seeds per capitulum ($F_{1,8.5} = 1.058$, $p = 0.332$), or average capitulum mass ($F_{1,8.7} = 3.829$, nearly significant decline at $p = 0.083$); for the last two results, log transformation of data improved normality but did not result in significance (log seeds per capitulum: $p = 0.464$; capitulum mass: $p = 0.057$). Corresponding ANOVAs also failed to identify differences among populations ($F_{10,144} < 1.6$; $p > 0.1$) except for reproductive traits: number of capitulae ($F_{10,144} = 2.390$, $p = 0.012$; Fig. 3A), average capitular mass ($F_{10,144} = 7.121$, $p < 0.001$; Welch's $p = 0.002$; Fig. 3B), and seeds per capitulum ($F_{10,144} = 3.838$, $p < 0.001$; Welch's $p = 0.062$; Fig. 3C) all varied among populations, and reproductive biomass approached significance despite a somewhat skewed distribution ($F_{10,144} = 1.565$, $p = 0.123$; Fig. 3D).

Finally, leaf toughness and trichome density could be determined only for non-withered plants.

Neither leaf toughness ($F_{1,8.9} = 0.078$; $p = 0.786$) nor trichome density ($F_{1,9.9} = 1.278$; $p = 0.285$) differed with ANCOVAs vs. latitude (Supplementary Figure S1). ANOVAs also failed to detect differences among populations for leaf toughness ($F_{10,99} = 0.773$, $p = 0.654$), but there was variation in trichome densities among populations ($F_{10,99} = 2.376$, $p = 0.015$).

Palatability Experiment

For both the *T. ni* and *C. nemoralis* palatability experiments, there were no significant differences among populations in percent leaf area remaining (*T. ni*: $F_{2,25} = 2.153$, $p = 0.137$; Figure 4A; *C. nemoralis*: $F_{2,44} = 1.408$, $p = 0.255$; Figure 4D) or leaf weight changes (*T. ni*: $F_{2,25} = 0.957$, $p = 0.398$; Figure 4B; *C. nemoralis*: $F_{2,44} = 2.399$, $p = 0.103$; Figure 4E). However, there were significant differences in herbivore weight change for both experiments (*T. ni*: $F_{2,25} = 6.274$, $p = 0.006$; Figure 4C; *C. nemoralis*: $F_{2,44} = 3.839$, $p = 0.029$; Figure 4F). *Trichoplusia ni* and *C. nemoralis* gained weight the most when they fed on leaf disks from Cochrane and the least when they were exposed to Newmarket leaf disks (Figures 4C, F). Snail residuals were non-normal due to one large outlier; excluding this outlier further improved significance ($p < 0.001$). Control leaf disks showed that there was not any significant difference in leaf weight loss due to water loss among populations ($F_{2,27} = 0.301$, $p = 0.743$).

Freezing Tolerance Experiment

There were totals of 82, 104, 109, and 114 *M. lappella* larvae found in the -24C, -29C, -34C, and -44C treatment groups. Larval survival differed among treatments (Pearson $X^2_3 = 149$; $p < 0.001$): a much greater proportion of larvae survived the -24C (94%) and -29C (88%) treatments than the -34C (28%) and -44C (34%) treatments (Figure 5).

Discussion

Damage to plants by their natural enemies may be reduced at marginal sites (Bruehlheide and Scheidel 1999; Alexander et al. 2007; Menéndez et al. 2008; Lakeman-Fraser and Ewers 2013); for

instance, Woods et al. (2012) found leaf damage to *Asclepias syriaca* (common milkweed) was greater in central than marginal populations. Previous work on *Arctium minus* showed a decrease in herbivory and improved performance in populations approaching the northern range limit of this plant (Kambo and Kotanen 2014). Latitudinal local adaptation may rapidly evolve in invasive species (e.g., Maron et al. 2004; Colautti and Barrett 2013). Nonetheless, our results indicate that, although there is some evidence of population differentiation, these latitudinal patterns are more likely to result from environmentally-driven gradients in exposure to herbivores than from genetic clines in plant traits.

Latitudinal trends in herbivore damage

Preliminary sampling of our common garden during August 2011 found very low levels of leaf damage, and non-significant differences among plants from different populations. This was expected because plants were transplanted late in the season; as a result, they had only a short period of time to accumulate damage. By August 2012, differences in folivory had developed among populations, but still no latitudinal trends were detected among source populations in folivory or damage by *Metzneria* to capitulae of the now-flowering plants. Damage levels were substantially higher than in 2011, though rates of folivory in our common garden still were much lower (1/10) than averages reported for wild populations near this site by Kambo and Kotanen (2014). Leaf miners were notably absent, though this alone does not explain low levels of leaf damage: leaf miners typically affect much less leaf area than chewing insects (Kambo and Kotanen 2014). Detection of trends may have been hampered by these low rates of folivory, but such rates of leaf damage do naturally occur in more northern regions, while damage by the important seed predator *Metzneria* was much more representative of many wild populations (~40%-90% of seeds destroyed in southern sites: Kambo and Kotanen 2014). The biennial life history of burdock meant that this experiment could not be extended beyond two years, but the fact that evidence of differences in leaf damage could be detected among source populations, and that many of our plants exhibited damage comparable to that reported for some wild populations (particularly for

Metzneria) suggest that sufficient time should have elapsed for any evidence of herbivore resistance to have become apparent.

These results contrast strongly with the striking latitudinal decline in damage documented by Kambo and Kotanen (2014). This indicates that the gradient of herbivore damage in wild populations is likely not due to genetic differences amongst populations; instead, burdock-feeding herbivores may be less abundant or active in northern areas. For example, Kambo and Kotanen (2014) documented declines in numbers of leaf miners with latitude; it is possible per-capita feeding also declined. These results also rule out the alternative possibility that northern populations have responded to locally reduced herbivory by investing fewer resources in resistance, as predicted by EICA hypothesis (Blossey and Nötzold 1995).

There are two general classes of mechanism that could produce such a pattern in herbivore abundance. First, herbivores might not be available in otherwise suitable locations, for instance if northern populations were too new to have yet been colonized, too small to support stable populations, or too isolated to yet have been located. Some anecdotal evidence might support the idea that burdock might be expanding in range faster than its enemies. For instance, Kambo and Kotanen (2014) found populations well to the north of those reported by Gross et al. (1980), while examination of herbarium specimens at the Royal Ontario Museum herbarium (2012) showed that there was an approximately 60 year gap between the earliest local record of burdock (1877) and that of *Liriomyza arctii* (1940) and *Calcomyza flavinotum* (1935). Herbarium records are imperfect evidence, but this still might suggest that the appearance of *Arctium* at a new location substantially preceded the arrival of its main herbivores; as well, rates of colonization by folivores in our experiment were slow. In particular, leaf miners were absent, despite their local abundance in natural populations (Kambo and Kotanen 2014). However, several lines of evidence argue that these explanations are unsatisfactory. Burdock has occurred in North America since at least 1638 (Gross et al. 1980), and may have occurred in northern

areas from a very early date: it is abundant at Moose Factory on James Bay (51°N); founded by the Hudson Bay Company in 1673, this is one of the oldest towns in Ontario. As well, northern populations are not necessarily small: though population boundaries often are difficult to define, Kambo and Kotanen (2014) found population size actually slightly increased with latitude. Finally, *Metzneria lappella* co-disperses inside the capitulum of its host, while both *Liriomyza arctii* and *Calycomyza flavinotum* (and presumably many other folivores) are native species presumably not restricted to *Arctium*; for instance, *C. flavinotum* also feeds on the widespread species *Eupatorium maculatum* (Spencer 1986). Consequently, it is unlikely that small size or isolation of burdock populations provides long-term protection from discovery by herbivores, especially if alternative hosts are present.

Alternatively, northern sites might be unable to support populations of herbivores even if they are able to reach those locations, likely because of inappropriate climate and/or photoperiod. For instance, herbivores may have a more limited climatic tolerance than their host plants (e.g., the butterfly *Aricia agestis* and its host *Geranium molle*: Pateman et al. 2012); though preliminary, our freezing tolerance experiment indicates this may be true for *Metzneria*. Climate also might operate indirectly to reduce herbivore success, for example by reducing plant growth or food quality; Kambo and Kotanen (2014) could find no clear evidence that more northern plants were less suitable hosts, but the existing evidence is limited.

Differences in performance among source populations

Our common garden detected no reproductive differences among populations, while Kambo and Kotanen (2014) showed greater viable seed production in northern populations. In part, their result reflected a reduction in damage by *Metzneria*, but it also reflected an absolute increase in fecundity. Our results indicate that the latitudinal gradient in seed production was likely due to trends in the biotic and/or abiotic environment of these plants, potentially including the latitudinal decline in folivory. Increased seed production at northern sites also might result from environmentally-dependent changes

in life history. Reinartz (1984) showed that the usually biennial common mullein (*Verbascum thapsus*) could spend two years or more in rosette form when environmental conditions were unfavourable, ultimately resulting in a fivefold increase in seed production (Reinartz 1984). In principle, harsh conditions might similarly extend the lifespan of *Arctium*, though this is not obviously supported by excavations of root systems.

Northern populations in our common garden had significantly higher rates of early withering and death, likely in response to the very hot summer of 2012. This suggests the possibility of local adaptation to climate: southern genotypes may have been selected for tolerance to hot, dry summers, while populations from cooler sites are more vulnerable to drought. Still, death is the normal fate of a biennial such as *Arctium* in its second year, and the timing of this event seems to have been affected more than other measures of performance. We detected no other evidence of latitudinal trends in plant health in our common garden: there was no trend in over-winter mortality, growth, or reproduction, and no trend in vulnerability to insects. If plants had been regularly watered, or if the summer had been more moderate, we likely would have seen better survival and performance, and may have seen higher rates of damage (notably by leaf miners); however, there is no reason to suspect that the effects of drought masked latitudinal trends in damage by leaf chewers or seed predators.

Herbivore resistance

Even though there were no correlations between latitude of origin and herbivore damage, there were still differences in folivory among populations. This indicates that, although they do not follow a latitudinal gradient, genetic differences in herbivore susceptibility may exist among populations; however, it is not certain what traits are involved.

There was a significant difference in the trichome density among populations. Trichomes can act as defensive structures (Schoonhoven et al. 2005); nonetheless, populations with greater trichome

densities did not have less damage. Trichomes can be induced by herbivore damage (e.g., Agrawal 1998; Traw and Dawson 2002); this may explain why Kambo and Kotanen (2014) found fewer trichomes at northern sites, where herbivores were scarce, while we noted no consistent trend in our common garden.

While chemical defences have not been studied in *Arctium*, our palatability experiment provides evidence that some populations may be better defended chemically. Herbivores grew less when they were exposed to plants from the Newmarket population compared to two more northern populations. Nonetheless, leaves of all three populations were consumed equally; these populations also showed similar herbivore damage in the common garden experiment. Since only three populations were tested, it is not possible to say whether these results represent a latitudinal trend.

Conclusions

Overall, our results indicate that the latitudinal gradients in leaf and seed damage found in previous work on *Arctium minus* (Kambo and Kotanen 2014) are not due to genetic differences, but instead are likely due to differences in herbivore abundance and/or activity with latitude. We did observe some differences in herbivory and possibly in defence among populations, but these were not clearly related to the latitudinal patterns of damage observed in the field. Instead, low levels of damage at northern sites apparently reflect escape, rather than defence. As a result, populations near the range limit of this invasive species apparently enjoy enemy release relative to heavily-damaged southern populations. This escape potentially could promote further range extension, for example, in response to climate change (e.g., Morriën et al 2010; Van der Putten et al. 2010). Lakeman-Fraser and Ewers (2013) provide evidence that such latitudinal enemy release may assist future expansion of a native shrub, *Macropiper excelsum*, in New Zealand: plants outside their current range perform better, likely because of the absence of a key lepidopteran herbivore. *Arctium* may represent an analogous case for an introduced species.

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Figure Legends

Fig. 1. Survival of burdock sampled from different locations, in a common garden experiment. 1A)

Risk of death before flowering vs. latitude. Regression analysis did not detect a significant trend ($p =$

0.505). 1B) Risk of early withering vs. latitude. Regression analysis found risk increased with latitude

of origin; shown is a linear fit ($p = 0.003$); for arcsin-transformed data, $p = 0.003$, $r^2 = 0.651$, $y' =$

$0.274x - 11.571$).

Fig. 2: Herbivore damage on burdock sampled from different locations, in a common garden

experiment (mean $\pm$ SEM). Neither ANOVA nor ANCOVA analysis detected any differences among

populations ($p > 0.2$, except as indicated). 2A) Proportion of leaf area damaged in August 2011. 2B)

Proportion of leaf area damaged in August 2012. An ANOVA analysis detected nearly significant

differences among populations ($p = 0.074$). 2C) Proportion of capitulae attacked by larvae. 2D)

Number of insect (primarily *Metzneria*) larvae occurring per capitulum during August 2012.

Fig. 3: Reproductive characteristics of burdock sampled from different locations, in a common garden

environment (mean $\pm$ SEM). ANCOVAs failed to detect any trends with latitude ($p > 0.2$ unless

indicated). 3A) Number of capitulae: ANOVA detected significant differences among populations ($p =$

0.012). 3B) Average capitular mass: ANOVA detected significant differences among populations ($p <$

0.001) and ANCOVA indicated a nearly significant latitudinal trend ($p = 0.083$, $r^2 = 0.323$, $y = -0.016x$

+ 1.000). 3C) Seeds per capitulum: ANOVA detected significant differences among populations ($p <$

0.001). 3D) Reproductive biomass: ANOVA found no significant differences among populations ($p =$

0.123).

Fig. 4: Results of palatability experiment (mean $\pm$ SEM). Different letters indicate significant differences ($p < 0.05$, Tukey-Kramer HSD). *Trichoplusia ni*: 4A) Percent of leaf disk area remaining. There was no significant difference among populations (ANOVA: $p = 0.137$). 4B) Leaf weight change. There was no significant difference among populations (ANOVA: $p = 0.398$). 4C) Larval weight change. There was a significant difference in weight gain depending on the treatments (ANOVA: $p = 0.006$). *Cepaea nemoralis*: 4D) Percent area remaining. There was no significant difference among populations (ANOVA: $p = 0.255$). 4E) Leaf weight change. There were no significant differences among populations (ANOVA: $p = 0.103$). 4F) Weight change of snails. There was a significant difference in weight gain depending on the population (ANOVA: $p = 0.029$).

Fig. 5: The proportion of seed eating moth (*M. lappella*) individuals that died when exposed to different temperature treatments. The four treatments differed significantly (chi-square: $p < 0.001$); bars sharing the same letter do not differ from one another ($p > 0.05$; Bonferroni-corrected X^2 tests).

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Supplementary Figure S1: Leaf characteristics of burdock sampled from different locations, in a common garden environment (mean $\pm$ SEM). S1A) Toughness (penetration force). Neither ANOVA nor ANCOVA analyses detected differences among populations ($p > 0.7$). S1B) Number of trichomes along 0.5 cm of main leaf vein. An ANCOVA found no significant difference in number of trichomes with latitude ($p = 0.285$), but ANOVA found differences among populations ($F_{10,99} = 2.376$, $p = 0.015$).