LETTER

Local adaptation primes cold-edge populations for range expansion but not warming-induced range shifts

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Abstract
According to theory, edge populations may be poised to expand species' ranges if they are locally adapted to extreme conditions, or ill-suited to colonise beyond-range habitat if their offspring are genetically and competitively inferior. We tested these contrasting predictions by transplanting low-, mid-, and high-elevation (edge) populations of an annual plant throughout and above its elevational distribution. Seed from poor-quality edge habitat (one of two transects) had inferior emergence, but edge seeds also had adaptive phenology (both transects). High-elevation plants flowered earlier, required less heat accumulation to mature seed, and so achieved higher lifetime fitness at and above the range edge. Experimental warming improved fitness above the range, but eliminated the advantage of local cold-edge populations, supporting recent models in which cold-adapted edge populations do not facilitate warming-induced range shifts. The highest above-range fitness was achieved by a 'super edge phenotype' from a neighbouring mountain, suggesting key adaptations exist regionally even if absent from local edge populations.

Keywords
Common garden, elevational gradients, experimental warming, life history trade-offs, local adaptation, offspring quality, phenology, range limits, reciprocal transplant, *Rhinanthus minor*.

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INTRODUCTION

Decades of theory exploring the ecological and evolutionary process limiting species distributions yield contrasting predictions about range-edge populations. On the one hand, species are thought to spread along environmental gradients via local adaptation at range margins (Levin 2000). Along a continuous gradient, adaptation to range-edge conditions should confer an advantage beyond the range, priming edge populations to expand the species' niche and range, and potentially facilitating range shifts in response to climate change (Hunter & Hutchinson 1994; Gibson *et al.* 2009). However, theory explaining stable range limits proposes that environmental gradients reduce fitness and population size towards the range edge (Sexton *et al.* 2009), an assumption increasingly supported by empirical data (Pironon *et al.* 2017). Under this scenario, offspring from small, isolated edge populations in harsh environments could suffer the negative effects of genetic drift, inbreeding, and poor maternal provisioning. Reduced offspring quality could thwart adaptation, reduce colonisation ability, and reinforce range limits (Brown *et al.* 1996; Hargreaves *et al.* 2014). Thus, while edge individuals are most likely to disperse beyond the range, whether they are the best or worst suited to colonise beyond-range habitat is unclear (McLane & Aitken 2012).

High-elevation and polar range edges are often associated with cold climate, such that local adaptation to climate enables poleward invasions (e.g. Colautti & Barrett 2013), and potentially facilitated range expansion after pre-historic glacial retreats (Cwynar & MacDonald 1987). Whether local adaptation to climate will facilitate range shifts driven by anthropogenic climate change is less clear (Bocedi *et al.* 2013;

Hargreaves *et al.* 2015a). Adaptation to a shifting climate gradient should favour range-edge genotypes as long as beyond-range conditions continue to resemble the range edge more than the range centre (i.e. dispersal keeps pace with climate change). However, if warming outpaces dispersal cool-adapted edge populations may be less suited to colonise newly-warmed habitat than central genotypes, slowing or stalling range shifts (Atkins & Travis 2010). Cold adaptation can also undermine colonisation directly by reducing fecundity (Hoffmann & Blows 1994); cold-adapted plants often reproduce earlier but at a smaller size, increasing their absolute fitness in short growing seasons but reducing their relative fitness in longer ones (Colautti & Barrett 2013). Of course, many ecological factors change towards high elevations and latitudes in concert with climate – establishing which limit fitness requires explicit experimental manipulations (Bjorkman *et al.* 2017).

Transplant experiments provide the best test of potential performance beyond a species range, local adaptation, relative offspring quality, and which life stages/ecological gradients impose the range limit (Hargreaves *et al.* 2014). Beyond-the-edge transplant experiments suggest many range limits are associated with declining habitat quality, though few confirm which habitat components constrain performance (Hargreaves *et al.* 2014; Lee-Yaw *et al.* 2016), and reciprocal transplants commonly find local adaptation within species ranges (Hereford 2009). However, few transplant experiments are designed to test for adaptation towards and beyond range edges, and the exceptions yield inconsistent results (Geber & Eckhart 2005; Stanton-Geddes *et al.* 2012; Samis *et al.* 2016). Moreover, many beyond-the-edge transplant experiments omit the early life history stages most related to offspring quality (Donohue 2009), and most do not replicate in time (multiple

generations of fitness) or space (multiple edge and beyond-range sites), increasing idiosyncratic year or site effects (Hargreaves *et al.* 2014). Critically, none have also included the climate manipulations needed to reveal edge populations' role in responding to climate change.

We tested the relative ability of edge populations to initiate upward range expansion using multi-year transplants and experimental warming. Along two elevational transects (Fig. 1), we reciprocally transplanted wild seed of the annual herb *Rhinanthus minor* L. throughout and above its elevational range to test for local adaptation, differing offspring quality, and their joint effects on beyond-range colonisation. We tested for the fitness gradients that underlie many range limits, predicting that: P1) habitat quality (i.e. lifetime fitness of local seeds) declines at the range edge, P2) lifetime fitness is too low to sustain populations beyond the range, and P3) low-quality edge habitat produces low-quality seeds (i.e. poor emergence across sites; Donohue 2009). We tested for adaptation to elevation, predicting that P4) high-elevation sources have the best lifetime fitness at and above the range edge, priming them for range expansion under current conditions. Finally, we monitored and manipulated climate to assess its importance in driving local adaptation and the range edge, predicting: P5) seeds from sites with cooler growing seasons need less heat accumulation to mature seed, P6) experimental warming would increase fitness at and above the range edge,

and P7) edge seeds would be best suited to colonise beyond-range habitat under warming.

MATERIALS AND METHODS

Study system

Rhinanthus minor (Orobanchaceae) is native to gently disturbed meadows of Europe and North America (Westbury 2004). It is a generalist root hemiparasite, obtaining nutrients from >50 host species, especially grasses and legumes (Cameron *et al.* 2006). Stems produce leaves in pairs (Fig. S1). Each leaf node can produce either a secondary branch or a bud that can develop into a fruit, resulting in a structural trade-off between growth and reproduction. Flowers are bee (*Bombus* spp.) pollinated, but can produce a full complement of viable seeds – up to 18/fruit – by autonomous self-pollination (Hargreaves *et al.* 2015b). Seeds rarely disperse > 2 m (Westbury 2004), making edge populations critical for beyond-range colonisation, though wet seeds can adhere to deer hide for > 100 m, potentially enabling rare long-distance dispersal (Murphy, Hargreaves, Eckert unpublished data). Most importantly for our purposes, *R. minor* is an annual with little seed dormancy (see Supporting Information 1.6), so lifetime fitness can be measured annually and is closely related to population demography.
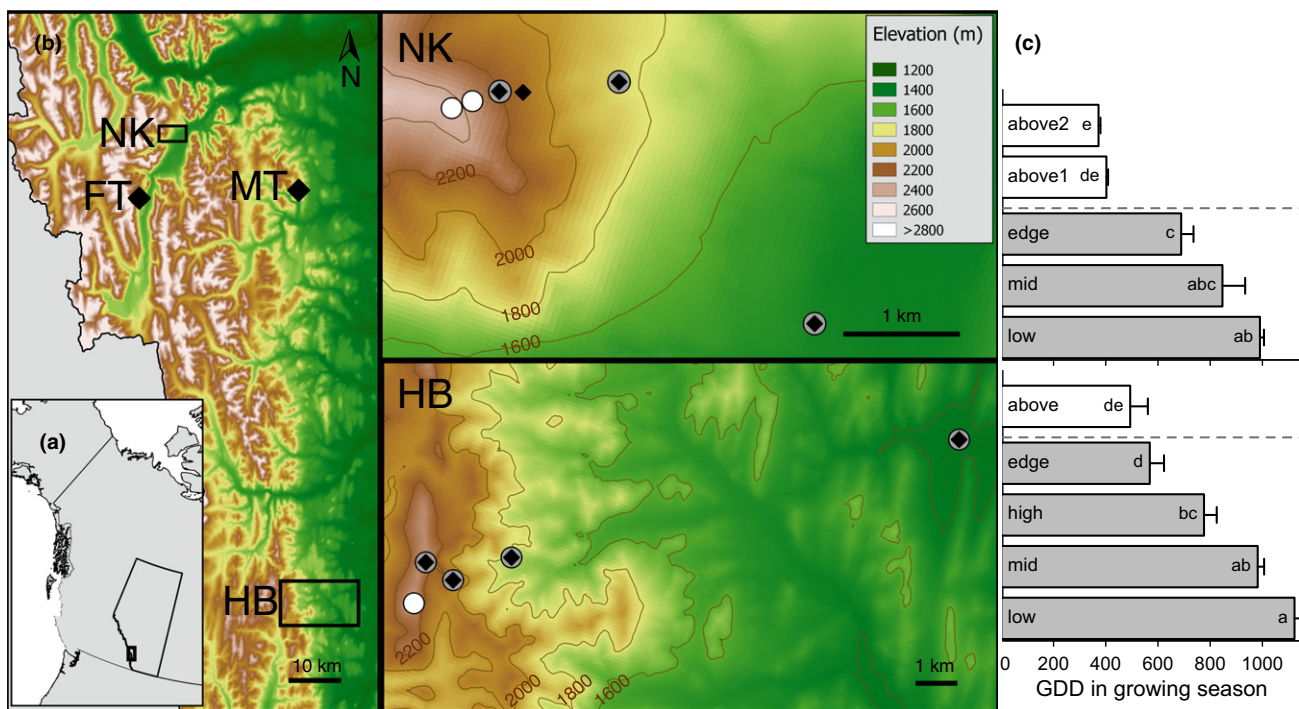

Fig. 1 Location and climate of field sites. (a) Thick rectangle shows study area within Alberta, Canada, enlarged in (b). (b) Elevation maps locating the four mountains that provided source material (left), with enlargements (right) showing reciprocal transplant transects: source populations (black diamonds), transplant sites within *R. minor*'s range (grey circles), transplant sites above *R. minor*'s upper range limit (white circles). (c) Mean ± SE heat accumulation per growing season at transplant sites during the 2011–2013 growing seasons. Dashed line indicates the upper range limit, contrasting letters indicate significant differences among sites and are comparable across transects (negative binomial GLM, likelihood ratio test of null model with intercept only vs. model with 'site' as a main effect; site $\chi^2_{df=1} = 73.7$, $P < 0.0001$).

We conducted transplant experiments along two transects, spanning consistent east-facing aspects of Nakiska (NK) and Hailstone butte (HB) peaks (Fig. 1, site descriptions in Table S1). In southwestern Alberta *R. minor* ranges from ~ 1200 to 2250 masl. At NK *R. minor* is relatively continuous up to ~ 2250 masl, whereas at HB the continuous range ends ~ 2200 masl, with an outlier population of < 30 plants at 2320 masl. Potentially suitable habitat (open areas with grasses or legumes) occurred above *R. minor*'s range on both transects. Populations were in protected but sometimes mixed-use areas. Along HB cattle graze up to 1800 masl, generally after *R. minor* sets seed, but fence failure gave cattle early access to HB-low in 2012 and HB-low and HB–mid in 2013, lowering fitness (Fig. S6).

Transplant design

Sample sizes varied among years but the core experiment design is straightforward (predictions in brackets): we reciprocally transplanted low-, mid-, and high-elevation seed throughout *R. minor*'s range to assess habitat quality (P1), offspring quality (P3), and local adaptation (P4-5); we transplanted these and additional high-elevation populations above the range to assess their colonisation potential (P2&4); and we experimentally warmed seedlings at and above the range edge to test whether heat accumulation limits beyond-range fitness (P6-7: Table S2 gives detailed yearly design and *n*).

Along each transect, we identified a source population from: below the elevational range center (Low), the elevational range center (Mid), and the upper range edge (Edge; source names capitalised throughout, Fig. 1). Edge populations produced too few seeds to supply reciprocal transplants without potentially affecting demography. We therefore planted Edge seeds into each edge site to assess home-source performance, but used seeds from the highest population > 2000 plants (High; 100 masl below the absolute range edge) for reciprocal transplants. Edge and High seeds generally had the same lifetime fitness (Fig. S7, Table S5), so we consider High seeds to accurately represent range-edge populations.

We established five transplant sites per transect (Fig. 1). Within each transect we transplanted Low, Mid, and High seeds into the low, mid, and edge sites, and the high population at HB (site names lower case throughout; Predictions1&3-5). We also established transplant sites in natural meadow above *R. minor*'s range; one at the highest elevation at that aspect (HB-above and NK-above2), and a site between that and *R. minor*'s range edge at NK (NK-above1; P2&4). HB-above was only 60 m above the range edge – i.e. no intermediate site existed – but had an alpine climate and plant community like NK-above2 due to its exposed, butte-top location (Figs S1A&S2). In contrast, HB-edge and NK-above1 were below rock outcrops that reduced wind but trapped snow, so had warmer but shorter growing seasons (Fig. S2) and grassier, subalpine vegetation. Populations of predominantly selfing species like *R. minor* may be genetically isolated from one another, trapping high-performing genotypes within individual populations (Samis *et al.* 2016), especially towards range limits (Eckert *et al.* 2008). To evaluate this possibility, we collected seeds from two additional high-elevation (2000 m) populations – Moose Mt (MT-High) and Fortress Mt (FT-High) – and planted them at high, edge, and above-range sites.

During August and September 2010–2012, we collected mature seed from 30 to 40 plants per source population (10–15 plants at HB-edge), > 1 m apart to reduce relatedness. Ten filled seeds/plant were assigned to each transplant site until the donor had contributed to all sites or all seeds were used. For each transplant site, seeds were pooled across maternal families within source population. Seeds were planted < 40 d after collection into 5–20 plots/site/year (Table S2), in grasses, sedges or legumes to provide suitable hosts. Before planting we removed naturally occurring *R. minor* in and around plots before they set seed. Each plot contained one subplot per source, of 25 seeds planted < 1 mm deep in a 25 × 25 cm grid (20 Edge seeds/subplot at HB-edge, as fewer seeds were available). Subplots were separated by ≥ 0.5 m, except in warming chambers due to space constraints. Apart from removing natural *R. minor*, vegetation was left intact.

Transplant design varied slightly between years. For 2011, we planted 10 plots/site in 2010. For 2012, five extra plots were planted at HB-low to offset losses from cattle, but snow covered HB-edge before plots could be planted. For 2013, sample sizes were reduced to five plots at low and mid sites (only 1.5 were successfully planted at HB-low due to soil compaction by cattle), and HB-edge and NK-above1 were not replanted. For 2013, we also planted five plots of Edge seeds at NK-above2 to compare their success to seeds from NK-High (insufficient HB-edge seeds were available to plant at HB-above). Sample sizes are fully described in Table S2.

Transplant monitoring

Plots were visited starting after snow melt (May–July) then once every 1–2 weeks. Individual plants were identified by their position on the planting grid, marked, and followed throughout their lives. Plants growing ≥ 2 cm 'off-grid' were considered potential contaminants and removed. For each subplot, we calculated the proportion of seeds to emerge and the proportion of emerged seedlings to produce viable seed. For each seed-producing plant we counted the viable fruits, viable seeds/fruit in ~ 25% of fruits/plant, total leaf nodes (i.e. final size), and estimated lifetime seed production (total fruits x mean seeds/fruit). Blackened or mouldy seeds never germinated in greenhouse trials and were considered inviable. Mean lifetime fitness was calculated per subplot as (total seeds produced)/(seeds planted).

We calculated three phenology parameters, interpolating between observation dates based on plant phenology at sequential visits. Emergence date was estimated per seedling as: date first seen – (#primary stem nodes x mean days to grow a primary stem node.) Growth rates were calculated at the plot level, accounting for faster growth at warmer sites. Start of flowering was estimated per plant as: date first seen flowering – 0.5 day per additional flower. We chose 0.5 as detailed observations (Hargreaves *et al.* 2015b) suggested two flowers open per day on average, and because it did not estimate dates earlier than the previous visit. Analyses on the observed start of flowering yield the same final models and significant contrasts (Table S6).

The date each source produced its first viable seed (seed maturation; monitoring was not precise enough to calculate this per plant) was estimated at each site based on the date seeds were first counted and notes on seed maturity (ie. maturity).

Experimental warming

After 2011 results suggested that cold, short summers severely limit *R. minor* reproduction above its range, we used open-topped warming chambers (OTCs) to test whether insufficient heat accumulation limits performance at and above the range edge (Fig. S1A; P6-7). Additional plots were planted for experimental warming in 2012 and 2013 at NK-edge, HB-high, NK-above2, and HB-above (Table S2). For 2012, we planted 10 plots of Mid and High seeds, local to their respective transect, per site. HB-High seeds had poor emergence in 2012; to ensure a sufficient sample size for the core local adaptation experiment at HB-high, we kept seven of the ten plots intended for OTCs in the control treatment instead, i.e. deployed only three OTCs. At HB-above we only deployed seven OTCs as three plots had no seedlings. To ensure a better sample size in 2013, we added a third source – MT-High – at all sites, and planted 10 extra plots of Mid and High seed at HB-above (Table S2). Aside from these extra HB-above plots, OTC-intended plots are paired with a control plot, planted side-by-side in similar vegetation and terrain. Once emergence began, we secured OTCs – clear plastic cones 0.4 m tall × 1.2 m diameter base × 1 m diameter opening – around experimental plots (Supporting Information 1.4). Transplants were monitored as above, and post-emergence fitness calculated as seeds produced/emerged seedling.

Climate monitoring

We measured temperature at plant height (2 cm above ground) using HOBO (Prov v2; Onset) and iButton (DS1921G; Maxim) sensors at each transplant site (details in Supporting Information 1.2; P5&6). From these data, we estimated the days of insulating snowpack, minimum winter temperature, growing season start and end date, and growing degree days (GDD) with $T_{base=10°C}$ and $T\mathrm{max}=30°C$ per growing season (Supporting Information 1.2). Sensor destruction by lightning and animals prevented calculation of all parameters in all site-year combinations. Additional iButtons were used in 2012 and 2013 to compare control vs. warmed plots at experimental warming sites ($n = 45$ iButtons/treatment across sites and years).

We compared climate among years using data from permanent weather stations. We compared above-range climate among our study years using air-temperature records from provincial or university-owned weather stations at the same elevation and < 1 km from NK-above2 or HB-above transplants. We assessed whether temperatures during our study were typical using long-term records from federal weather stations < 20 km from our sites (Supporting Information 1.3).

Analyses

Performance and phenology analyses consider subplot as the unit of replication, accounting for non-independence of seeds within subplots. Ratios (seeds to emerge, emerged seedlings to reproduce, seeds produced/emerged seedling and seeds produced/seeds planted) are calculated per subplot. As warming manipulations began after emergence, emergence analyses include data from warmed and control plots when both are available. Analyses of lifetime seed production (seeds/reproductive plant) and phenology use subplot means.

Performance and phenology were analysed by transect. In-range performance models considered home-transect sources (Low, Mid, High) at in-range sites (low, mid, edge for NK; low, mid, high, edge for HB; Predictions1&3). Full models considered all possible interactions: ∼ Elevation × Source × Year for NK; ∼ Elevation × Source + Source × Year for HB as Source and Year were not fully crossed. Analyses of performance across the range limit (P2&4) including home-transect sources (Low, Mid, High), foreign high-elevation sources (MT-High, FT-High), and sites spanning the range limit (NK-edge, NK-above1, NK-above2; HB-high, HB-edge, HB-above) from 2011 to 2013. Elevation and Year are not fully crossed as NK-above1 and HB-edge sites were not planted every year, so the full model is: ∼ Elevation × Source + Source × Year). Plants often failed to reproduce above the range, so we did not analyse seed production/reproductive plant across the range limit. Phenology analyses included home-transect sources at all sites. All models include a random intercept for plot and an individual-level random intercept, 'subplot', to account for overdispersion (Bolker 2015).

We used generalised linear mixed models (GLMMs; *glmer*, lme4 package (Bates *et al*. 2015)) in R 3.3.3 (R Development Core Team 2015)). Error distributions were binomial for proportional parameters (logit link function), and Poisson for seed count and phenology parameters (log link). Term significance was assessed by comparing models with and without a given term using a likelihood ratio test and χ^2 distribution. Non-significant ($P > 0.05$) effects were retained in the model if integral to the experimental design ('Elevation', 'Source'), but dropped otherwise (interactions, 'Year'). An alternate approach when Year and Elevation are not fully crossed is to run one model with all sites and some years, and a second with all years and some sites; this approach yields the same significant contrasts (not shown). We tested differences among levels using least squared means with the Tukey method to maintain $\alpha = 0.05$ (*lsmeans*, lsmeans package 2.25 (Lenth 2016)). For interacting predictors, pairwise tests were conducted within levels of the interacting term. If performance was zero for one site (i.e. failure above the range), significant site differences were identified when the back-transformed lower 95% confidence limit for other sites was > 0.

Warming experiment analyses considered transects together, site-elevations grouped as 'near the edge' (NK-edge, HB-high) or 'above the range' (NK-above2, HB-above; P6&7), and sources in the warming experiment each year (local Mid and High sources for 2012; Mid, High and MT-High for 2013). We were not interested in year effects unless they affected the warming treatment, so we included Year as a fixed effect that interacted only with warming treatment (modelling Year as a random intercept yields the same final models and significant contrasts). We used GLMMs with random intercepts for plot

pair (i.e. paired control and OTC plots) and 'subplotID'; full model: performance ~ Warming x Elevation.grouped x Source x Transect + (1|plotpairID) + (1|suplotID). Interactions, Transect, and Year were dropped from models if non-significant in likelihood ratio tests. As warming began after emergence, analyses compare post-emergence performance (seeds/emerged seedling), i.e. the maximum possible mean lifetime fitness if all seeds emerged. Intermediate performance and phenology parameters given in Table S9.

To assess persistence at and above the range edge (P2), we calculated density-independent population growth rates (λ) using matrix models (Caswell 1989; details in Supporting Information 1.5). Models used across-year means for emergence and seeds-per-emerged-seedling for each source-site combination, and the transect mean for dormancy (i.e. across the three highest sites and all sources and years) as dormancy did not differ among sites or sources (Supporting Information 1.6). To test whether warmer growing seasons would make above-range habitat suitable (P6-7), we estimated λ using post-emergence performance from the warming treatment and emergence data either local to each site (i.e. assuming warming does not affect emergence) or from the range edge (i.e. warmer growing seasons plus improved over-winter conditions).

Climate analyses used negative binomial generalised linear models to compare GDD/season among sites, and linear mixed models, with plot ID as a random intercept, to compare temperature and GDD/day between control and warmed plots (Supporting Information 1.4).

RESULTS

All performance measures declined above *R. minor*'s range along both transects (Figs 2 and 3, Table S7), even though fitness of local sources did not always decline from the range centre to edge (Fig. 3, Table S8). When local fitness did decline at high elevations, indicating low-quality edge habitat (Fig. 3 for HB transect), edge seeds were also low-quality, with poor emergence across sites (Fig. 2a for HB). Lifetime fitness was too low for self-sustaining populations above the range, and at the outlier population defining the range edge on the HB transect (Fig. 3). Even accounting for seed dormancy, these sites had negative estimated population growth rates ($\lambda < 1$; Fig. S5).

The sharpest performance decline at high elevations was the proportion of emerged seedlings that matured seed (Fig. 2b). All source populations failed to reproduce in at least 1 year at every above-range site (Fig. S8). This was not due to poor survival, as longevity did not decline from range edge to above-range sites (Fig. S9). Rather, many plants stayed small and never flowered, a phenomenon never seen within the range. Plants that did make reproductive structures initiated them late (Fig. 4), often losing some or all to frost or snow damage (Fig. S1E).

Source populations differed in phenology, imparting low- and high-elevation sources a home-site advantage consistent with local adaptation at one transect (HB). High-elevation plants generally flowered first (Fig. 4, Table S6) – sometimes finishing flowering before low-elevation plants began – so were the most likely to mature seed at all sites within the range (Fig. 2b). In contrast, plants from low-elevation seed delayed flowering to grow secondary branches; this increased both their potential fruit production and the heat accumulation they required to produce seed (mean GDD $\pm$ SE: NK-Low 590 $\pm$ 16, HB-Low 552 $\pm$ 20) compared to high-elevation plants from the same transect (NK-High 521 $\pm$ 20, HB-High 468 $\pm$ 18; effect of source: NK $\chi^2_{d.f.=1}$ 7.7, $P < 0.05$, HB $\chi^2_{d.f.=1}$ 10.7, $P < 0.01$, Fig. S10). Low-elevation plants thus produced more seeds given enough GDD (Fig. 2c), but high sources had greater lifetime fitness in the predictably shorter summers at high elevations (Fig. 3) and in growing seasons truncated by drought or cattle at lower elevations (Fig. S8).

High-elevation sources, both local to each transect and from adjacent mountains, enjoyed the best performance above *R. minor*'s range. High-elevation plants outperformed low-elevation, and often mid-elevation plants in lifetime fitness (Fig. 3) and population growth rates (Fig. S5) at all three above-range sites. Indeed, none of > 2000 low-elevation seeds planted at the highest above-range sites made seed (Fig. 2c).

Growing season GDD was the only climate variable that declined across the range limit in concert with lifetime fitness (Fig. 1c; other climate variables shown in Fig. S2). Growing season warmth decreased by 60% from the lowest to highest sites (Fig. 1c). The four sites above 2300 masl, where *R. minor* $\lambda < 1$, accumulated significantly fewer GDD than sites < 2300 masl where transplanted populations were self-sustaining (Fig. 1c). Natural temperature variation among years also suggests that growing season warmth limits fitness above *R. minor*'s range. Compared to long-term averages, July and August 2012 and 2013 were unusually warm above the range at NK (Fig. S3) and seedlings from multiple sources matured seed, whereas none matured seed in the more typical summer of 2011. In the warmest (2013) vs. coldest (2011) growing season of our study, there were 25% more GDD above the range at both transects (Table S4), and > 30 times more transplants matured seed (9 of < 3000 seeds above the range in 2013 vs. 1 of > 7000 in 2011).

Experimental warming confirmed that inadequate heat accumulation during the growing season limits *R. minor*'s fitness above its range, but eliminated the advantage of high-elevation seeds. Chambers warmed the air by 1.1 $\pm$ 0.18 °C, adding 1.4 $\pm$ 0.43 GDD per day (mean $\pm$ SD, Fig. S4; see Supporting Information 1.4 and 3 for full discussion of OTC effects). This is roughly the temperature equivalent of descending to the next highest site (Fig S4), so if elevational fitness patterns reflect temperature, fitness in OTCs should resemble fitness at the site below (i.e. Fig. 3). Consistent with this prediction, warming increased performance (seeds/emerged seedling) above *R. minor*'s range, but not enough to make populations viable, and improved performance at the HB-high but not NK-edge site (Fig. 5). The benefit of warming was derived mainly from an increase in the proportion of plants able to flower rather than earlier flowering (Table S9). Warming particularly improved the performance of mid-elevation plants (Table S9); in contrast to the overall results (Fig. 3), in the warming experiment all sources had equivalent performance above the range (Fig. 5).
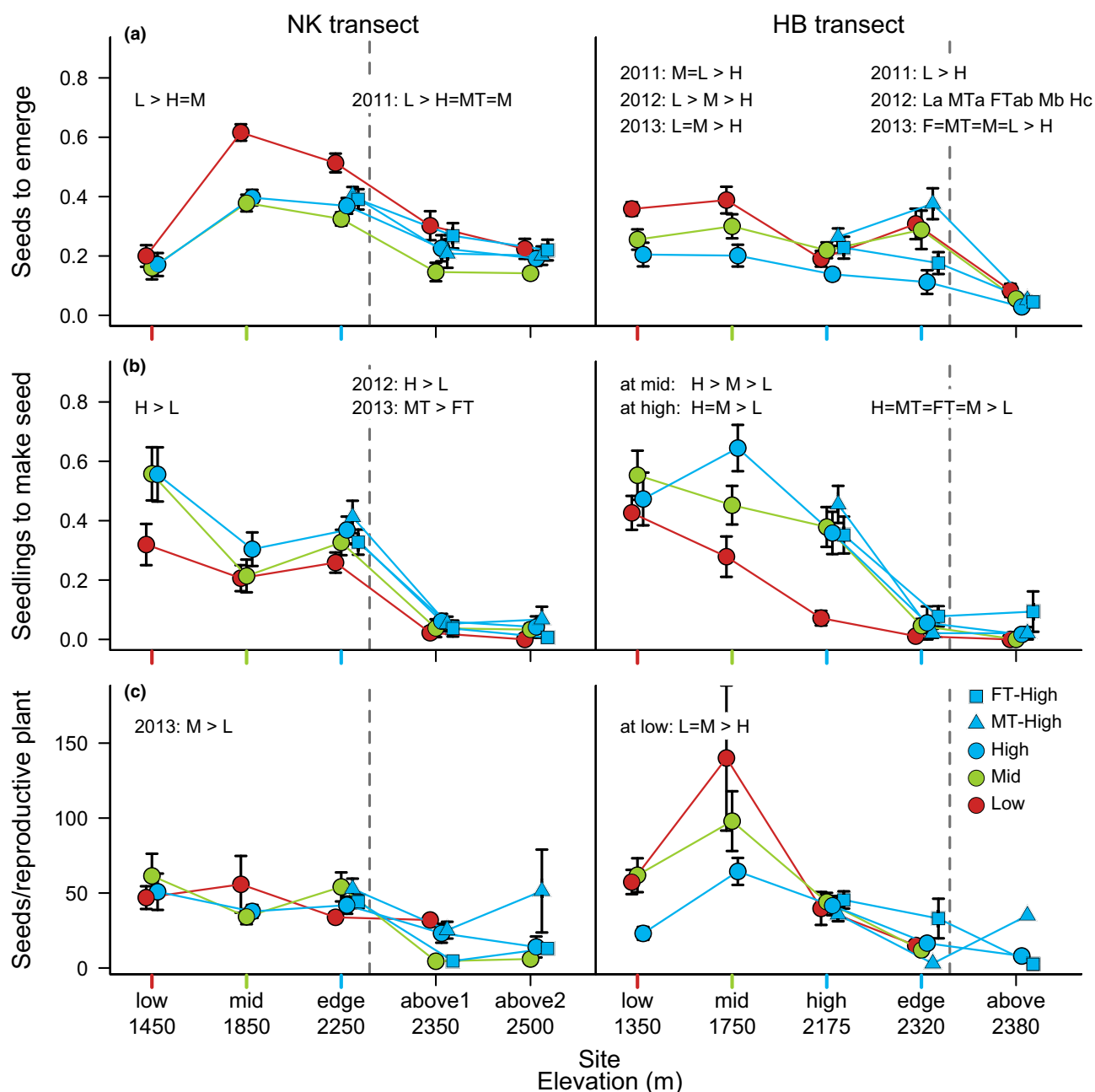
Fig. 2 Performance of seed from different elevations within and above *R. minor*'s elevational range. Each performance parameter at each transect is analysed as two experiments: (1) performance at sites within the range (left of dashed line) by local low-elevation (red circles), mid-elevation (green circles), and high-elevation (blue circles) sources, *x*-axis tick colour indicates the most local source population for each site; (2) performance across the high-elevation range limit (dashed line) including the highest three sites on each transect, comparing seeds from local sources and neighbouring high-elevation populations (blue squares and triangles). Text indicates significant differences among sources in the range (left justified) and across the range limit (spanning dashed line), separated by year and/or site if the final model contained significant Source x Year or Source x Site-Elevation interactions. Full statistical results in Tables S7 and S8. Points are mean ± SE of performance across 3 yearly cohorts (2011, 2012 and 2013) for all sites except HB-edge (2011 only) and NK-above1 (2011 and 2012 only).

DISCUSSION

Theory predicts that local adaptation will involve traits associated with the environmental gradients that limit fitness at and beyond range edges, and will prime edge populations to colonise beyond-range habitat (Hoffmann & Blows 1994;

Hargreaves *et al.* 2014). Growing season warmth was an important gradient underlying *R. minor*'s high-elevation range limit; the coldest sites and years had the lowest fitness, and experimental warming significantly improved fitness above the upper range edge (Fig. 5). Fitness above *R. minor*'s range was severely limited by reproduction, with
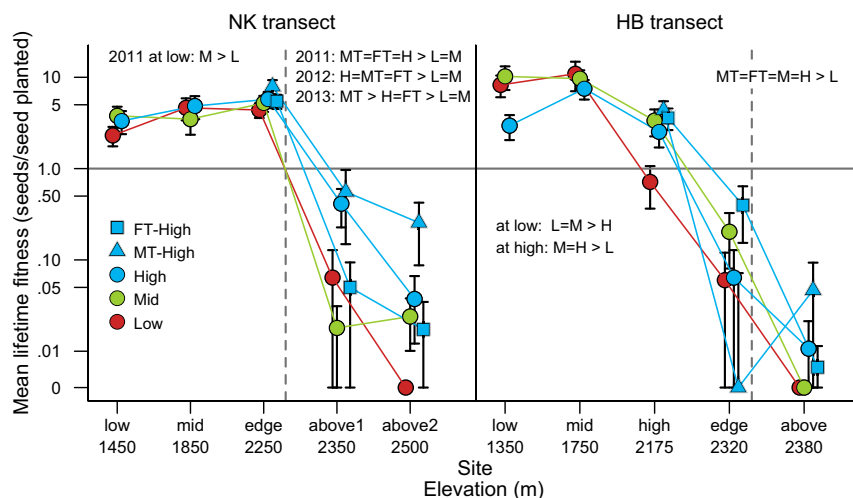
Fig. 3 Mean ± SE annual lifetime fitness from 2011 to 2013 in and above *R. minor*'s elevational range. Text shows significant differences among source populations. At NK, source differences in performance at component life history stages (Fig. 2) did not result in local adaptation (i.e. a significant Site-Elevation x Source interaction) within the range, but high-elevation sources (blue) significantly outperformed mid- and low-elevation sources at and above the range limit. At HB, sources showed significant local adaptation within the range (Site-Elevation x Source interaction $\chi^2_{df=4}$ 25.3, $P < 0.001$, left-hand text), which was broadly reflected in performance across the upper range limit. Formatting as per Fig. 2, full statistical results in Tables S7 and S8.

complete failure in some years, imposing severe viability constraints for an annual plant with low seed dormancy. As predicted, high-elevation plants had traits associated with reproducing in cool, short growing seasons. High-elevation plants required the fewest GDD to produce seed (Fig. S10), conferring a fitness advantage at and above the range edge (Fig. 3). While non-temperature gradients could also reduce fitness above *R. minor*'s range, they cannot easily explain local adaptation in phenology or the positive response to experimental warming. Our results support mounting evidence that climate commonly limits cold range edges, often via phenological constraints rather than cold tolerance *per se* (Griffith & Watson 2005; Colautti & Barrett 2013; Hargreaves *et al.* 2014).

High-elevation populations had the best lifetime fitness at high elevations, despite having lower emergence success at one transect (HB). The possibility that range-edge offspring are simultaneously poor quality at early life stages and locally adapted later has been overlooked in the extensive theory on species distributions. In retrospect, their co-occurrence may be common since local adaptation and poor offspring quality arise from the same gradient in habitat quality. Interacting effects of poor genetic quality and local adaptation may result in counter-intuitive evolutionary dynamics at range edges (Gilbert *et al.* 2017).

Reproductive phenology is a consistently heritable trait (Hendry & Day 2005) commonly involved in adaptation to climate (Colautti & Barrett 2013), but could be influenced by non-heritable adaptive maternal effects as well. Any local advantage due solely to adaptive maternal effects would be short-lived (e.g. lower-elevation genotypes growing at high elevations would produce offspring with high-elevation phenology). However, we feel that genetic adaptation is a more likely explanation for adaptive phenology. Theoretically,

conditions that are consistent across the scales of pollen and seed dispersal, such as large elevational differences, should promote local adaptation instead of adaptive maternal effects (Galloway 2005). Empirically, the best current review suggests adaptive maternal effects (i.e. those that would mimic local adaptation, not overall provisioning/offspring quality) are generally uncommon and weak (Uller *et al.* 2013). Ecologically, the distinction is less important: whether heritable or not, edge populations were the most likely and best able to colonise beyond-range habitat and expand the species range.

The colonisation advantage of local adaptation, however, was inconsistent. Although high-elevation sources outperformed lower-elevation sources above the range (Fig. 3), local high seeds lost this advantage under warming. When above-range plots were warmed, mid-elevation seeds had numerically (though not statistically) higher post-emergence performance (Fig. 5) and estimated population growth rates (Fig. S5) than local high-elevation genotypes. Mean temperatures (Fig. S4) and fitness (Fig. 5) under warming were still lower than at the range edge, so the advantage of range-centre seeds was not because OTCs recreated range-centre conditions. Local high-elevation seeds also lost their advantage in the unwarmed control treatment (Fig. 5), contrasting their overall superiority at high elevations (Fig. 3). This discrepancy arises because the control treatment excludes data from years and sites where local high seeds most outperformed mid seeds: 2011 (which was colder than 2012–2013), and the HB-edge and NK-above1 sites (performance was just high enough for a source advantage to be expressed in lifetime fitness, compared to the extremely low fitness at the highest sites; Fig. S8). The advantage of local edge populations was thus ephemeral, manifesting primarily in extreme environments, and disappearing with even slight amelioration of the fitness-limiting gradient. These
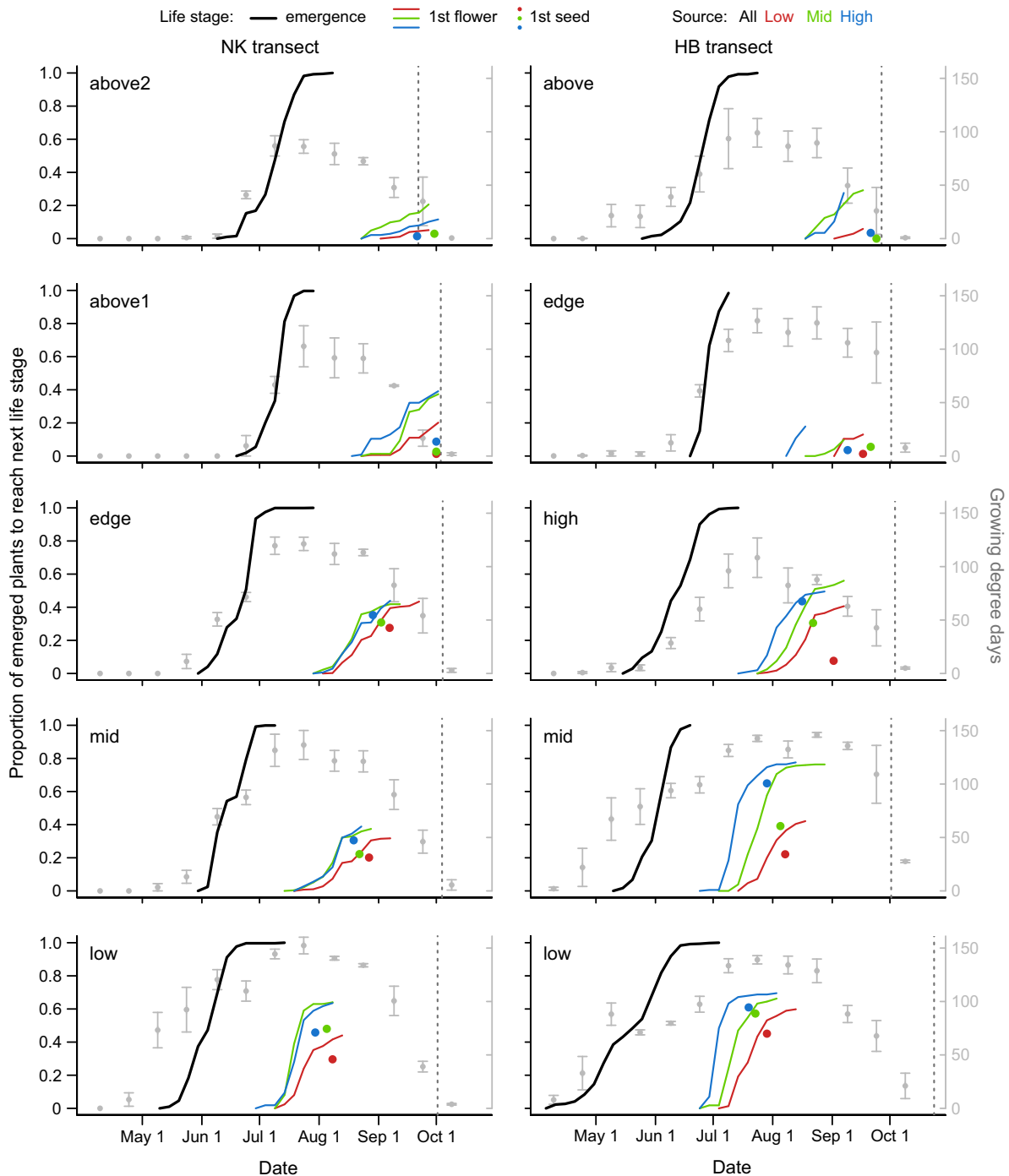
Fig. 4 Phenology of plants from contrasting elevations transplanted across and above *R. minor*'s range. Seeds were from low-, mid- and high-elevation populations local to each transect, transplanted to sites within (low to upper range edge) and above the elevational range. Curves show the accumulation of individual plants reaching a given life stage for 2011–2013 combined (2011–12 only for NK-above1, 2011 only for HB-edge), but models consider subplot means. Source populations did not differ in mean emergence date at any site so emergence curves combine data across sources (likelihood ratio tests, source contrasts $P \geq 0.1$, Table S5). Sources differed significantly in mean date of first flower both within and above the range. At NK plants from mid- and high-elevation seed flowered earlier than plants from low-elevation seed across sites (likelihood ratio tests, source $P < 0.001$ for each year). At HB, high-elevation plants flowered earlier than mid-elevation plants which flowered earlier than low-elevation plants across sites (likelihood ratio tests, source $P < 0.001$ for each year). Seed maturation was not monitored precisely enough to generate phenology curves, so coloured points show the average date of first seed maturation across years for each source. Low points are missing from top panels as no low-elevation plants matured seed at the two highest sites. Seed maturation dates for NK-above1 are from 2012 only, as no seed was produced in 2011; all viable seeds were found during the last check in 2012 and differences in seed colour (i.e. time since maturation) were not recorded, so sources have the same estimated seed maturation date. Grey points show heat accumulation (mean ± SE GDD for up to 5 years, 2010–2014) during each half of each month, dotted lines show mean estimated end of growing season defined by 3 days of consecutive snow pack or 2 + h of −4 °C or colder. Date of emergence and first flower differ among sites within transects, getting progressively later as elevation increases (Table S6).
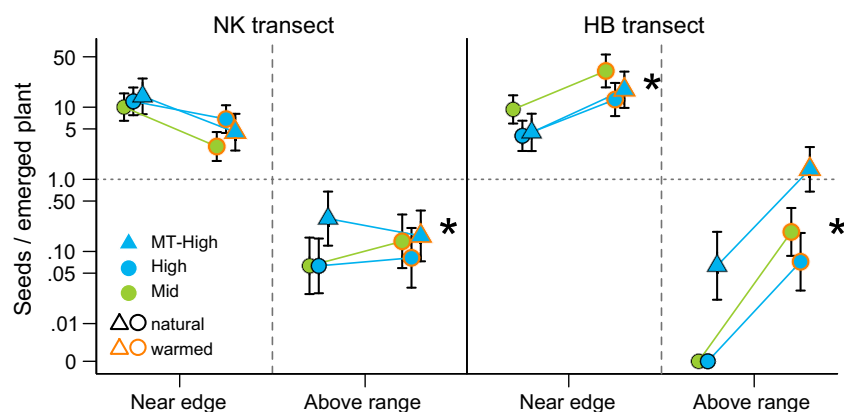
Fig. 5 Effect of experimental warming. Plants in warmed plots outperformed those growing in natural climate conditions above the range at both transects, and close to the range edge at HB. Analyses grouped site-elevations as 'near edge' [NK-edge (2250 m) and HB-high (2175 m)], or 'above range' (NK-above2 (2500 m) and HB-above (2380 m)); vertical dashed lines denote upper range limit. * denotes sites where the effect of warming was significant: above the range but not at the range edge (Warming × Site-Elevation interaction: likelihood ratio test, $\chi^2_{d.f.=1}$ 5.4, $P = 0.020$) and at the HB transect but not the NK transect (Warming x Transect: $\chi^2_{d.f.=1}$ 25.3, $P < 0.001$). Seeds from the high population on neighbouring Moose Mountain outperformed local high and mid-elevation seeds above the range (Site-Elevation x Source: $\chi^2_{d.f.=2}$ 12.9, $P = 0.0016$). Points show least squared means $\pm$ SE back-transformed from the simplest model with the 4-way interaction: Warming × Site-Elevation × Source × Transect + Year + (1|plotpair) + (1|subplotID).

results support results from simulation models showing that local adaptation to cool climate may not favour range-edge populations in warming-induced range shifts (Atkins & Travis 2010), though climate change effects will be more complex than simple warming (discussed in S1 3).

In contrast to the ephemeral advantage of local high-edge seeds, high-elevation seeds from neighbouring Moose Mountain outperformed other sources in lifetime fitness (Fig. 3) and growth rates (Fig. S5) at five of the six highest sites, and in both natural and warmed plots of the warming experiment (Fig. 5). Based on theory, the success of this population was unexpected; it was not larger, less isolated, different climatically, or more fecund at its home site than other high-elevation populations (Table S1). Although separated from other populations by < 100 km (Fig. 1) – a relatively modest distance in landscape terms – this 'super phenotype' was restricted to one of four mountains. The highlights that even widely beneficial adaptations may be unable to spread (Leimu & Fischer 2008), particularly among isolated populations (Samis *et al*. 2016). Genetic isolation of edge populations from each other likely plays an underappreciated role in stalling adaptation at range limits (Sexton *et al*. 2011).

While growing season warmth clearly constrains fitness across *R. minor*'s high-elevation range limit, other features of the elevational gradient may also play a role, though earlier experimental work rules out pollination deficits (Hargreaves *et al*. 2015b) and herbivory (Falk *et al*. 2013). Emergence declined across the range edge (Fig. 2A), implicating constraints acting between fall seed maturation and spring emergence, such as cold winter temperatures or high post-dispersal seed predation. We are often asked whether host plants limit *R. minor*'s distribution. *Rhinanthus minor* is a host generalist, host genera are found above its range, and transplants were always planted in patches of suitable hosts, so neither the range edge nor the fitness declines we detected are due to

categorical lack of hosts. The plant community does change across *R. minor*'s upper range edge, including declining relative abundance of legumes (Bocchinfuso *et al*. 2017), which are thought to be particularly valuable hosts. However, *R. minor* fitness did not covary with legume abundance at the plot scale (Bocchinfuso *et al*. 2017). Thus, while we cannot rule out a role for hosts in limiting *R. minor*'s range, we have no evidence for this to date (as opposed to the inferential and experiment evidence for climate), nor any reason to think below-ground interactions would be more important for a hemiparasite than for the >80% of vascular plants that obtain nutrients via mycorrhizal associations.

The conservation importance of range-edge populations is vigorously debated (Channell & Lomolino 2000; Gibson *et al*. 2009), and our results add important insights to this broader conversation. Edge populations are often valued for their putative colonisation potential, and we show this may be difficult to assess for any given population. Edge populations can harbour cryptic adaptations that might facilitate success beyond the range even in the absence of a home-site advantage at the range edge (NK transect). Superior populations may not be identifiable based on population size and isolation, parameters widely thought to determine population quality (Hoffmann *et al*. 2017; MT-High source). Finally, although edge populations were best suited for natural range expansion, local-edge populations lost their advantage over central genotypes under climate warming, thus their importance for range dynamics may be context dependent. More hopefully, the global success of some non-local edge genotypes suggests that gene flow between isolated edge populations could enhance fitness, and possibly adaptive potential, at and above the range edge (Sexton *et al*. 2011). Together, these results suggest that a regional approach to conserving isolated edge populations, potentially including enhanced gene flow among them, could maximise species' ability to respond to global change.

AUTHORSHIP

AH and CE designed the study together, AH collected and analysed the data, AH wrote the manuscript and both authors contributed substantially to revisions.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3bd420c

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