Supporting information for:

Germination traits largely do not evolve post-invasion: Comparisons in the native and introduced range of seven herbaceous plant species

Harold N. Eyster* & Elizabeth Wolkovich

*Corresponding author. Please direct any questions or comments to haroldeyster@gmail.com.

Contents

1 Additional general methods

1.1 Defining Invasive Species

There is no consensus on how to classify a species as invasive (Colautti & MacIsaac, 2004). The most common terms include 'exotic,' 'introduced,' 'naturalized,' 'nonindigenous,' 'established,' 'alien,' 'noxious,' 'weedy,' and 'invasive.' These terms can be grouped into those that describe the provenance of the species (e.g., exotic, introduced, alien, non-indigenous), those that describe its ability to grow and compete in the new ecosystem (e.g., naturalized, established), and those that describe its impact on the receiving ecosystem (e.g., noxious,

weedy, harmful). The International Union for the Conservation of Nature (IUCN, 2008) describes invasive species as: "organisms introduced by man [sic] into places out of their natural range of distribution, where they become established and disperse, generating a negative impact." However, this definition contains three subjective elements: what timepoint of a species' range is 'natural,' whether humans are a natural part of nature, and what is defined as a negative impact (Munro et al., 2019). To both acknowledge and allay some of these subjective elements, this paper follows the definition of Richardson et al. (2011), Richardson et al. (2000). Invasive species are thus those that (1) are introduced across a previously unpenetrated barrier, (2) successfully reproduce in the place of introduction to create a stable local population, and finally (3) spread to produce fit offspring a substantial distance from the place of introduction.

1.2 Study species details

Capsella bursa-pastoris (CAPBUR; Shepard's Purse) is an annual or biennial herbaceous plant in Brassicaceae. It grows 10 to 80 cm tall, typically blooming in late spring (Defelice, 2001). It originated in Europe, and was introduced to the New World as a medicinal herb—it is now found across Canada, the US, and Mexico (Westrich, 1989).

Chelidonium majus (CHEMAJ; Greater Celandine) is an herbaceous biennial member of Papaveraceae. It is native to Eurasia and North Africa and was introduced to the US by the 1670s as a medicinal. It is now found across the Eastern US and Canada and portions of the west (Holm et al., 1979).

Dactylis glomerata (DACGLO; Orchard Grass) is a cool-season, perennial grass (Poaceae). Plants grow up to 120 cm tall and have roots up to 60 cm long (Moser & Buxton, 1996). This plant originated in central and western Europe, and was intentionally introduced into the US in the 1750s (Bush et al., 2012) as a forage grass for pasture and hay (Ogle et al., 2011).

Plantago lanceolata (PLALAN; Narrow-leaved Plantain) is a perennial member of Plantaginaceae. It has narrow, ribbed leaves and grows to 1 m tall. It is native to Eurasia, and has successfully colonized the world's mid-latitudes (Holm et al., 1977).

Plantago major (PLAMAJ; Broad-leaved Plantain) is a perennial member of Plantaginaceae. It has broad, smooth leaves, and grows to 15 cm tall. Native to Europe, it was introduced into North America for its medicinal uses (Knobloch, 1996; Samuelsen, 2000).

Rumex crispus (RUMCRI; Curly Dock) is a perennial herbaceous plant in Polygonaceae, and grows to 160 cm. It is native to Europe, Asia, and Africa, and was introduced for its medicinal uses into North America where it now found across much of the continent (USDA, 2010).

Taraxacum officinale (TAROFF; Dandelion) is a perennial herbaceous plant in Asteraceae, and grows to 60 cm. It is native to Eurasia, but is now found in all 50 US states, much of Canada, and Mexico (USDA Agricultural Research Service, 1971).

1.3 Sampling details

Numbers of sampled populations and individual plants from which we collected seeds are given in Table S1. We used Bayesian multilevel models to account and control for greater

sampling in the native range.

Table S1: Total number of seed-producing individuals and populations from which seeds were collected.

US populations	US individuals	European populations	European individuals
3	21	13	63

1.4   Selecting period/light luminance

Some species have sufficient Pfr (the active form of phytochrome pigment, often necessary to induce germination) and so do not need any light to break dormancy, others just need a pulse of red light to break dormancy (the red light converts the inactive phytochrome into Pfr, Casal & Sánchez, 1998). Other species, including *Plantago major*, take much longer to build up the requisite Pfr, and so have much higher germination success when exposed to longer periods of light (with nearly 100% germination after 48 hours of exposure for *P. major*, Pons, 1991). Finally, some species have a high irradiance response (HIR), germinating poorly when exposed to high luminance light or prolonged light (Roberts et al., 1987). Beyond interspecific variation, there is also intraspecific variation in the relationship between dormancy and light (Probert et al., 1986). Across all populations, germination rate seems to be log-normally related to photon dosage (Ellis et al., 1986). Light may begin inhibiting germination for HIR species at about 0.1 mol/m^2/day–1 mol/m^2/day (Baskin & Baskin, 1998; Ellis et al., 1986), while other species peak above 10 mol/m^2/day (Ellis et al., 1986). The differing levels of light necessary to break dormancy means that any chosen light regime will be better for some species and worse for others.

The goal of these experiments was to create germination rates that are sufficient to observe variation in responses to treatments. Thus, we chose an intermediate light exposure at which all of the species would germinate at substantial levels, but which may not be ideal for any species. In selecting how much light to use, this experiment erred on the side of too much light rather than not enough, since at least one of our species is known to need large amounts of light (*Plantago major*, Pons, 1991), but none are known to exhibit HIR (although a *D. glomerata* subspecies in southern Europe does exhibit HIR, see Probert et al., 1986, however, this subspecies is not thought to have been collected for this study). Thus this experiment used a length of eight hours at a luminance of 75 micromol/m^2/second to yield a daily photon dosage of 2.16 mol/m^2. Baskin and Baskin (1998) recommend that the light period coincide with the high-temperature period. Thus, this experiment exposed seeds to eight hours of fluorescent light during the high-temperature thermoperiod.

1.5   Selecting substrate and planting depth

Substrate and planting depth can affect germination. Popay and Roberts (1970) show that *C. bursa-pastoris* seeds germinated about equally on filter paper as on top of soil, but showed

much decreased germination when inserted into soil. However, the effects of planting substrate and depth have not been studied in most of the study species. Nevertheless, a study on a species related to *D. glomerata* suggests that *D. glomerata* may germinate better in soil (Andrews & Burrows, 1974). Moreover, a difference in depth of just a couple millimeters can result in extreme differences in light availability, which can be essential for germination (Tester & Morris, 1987). Thus each seed was placed on top of Fafard Growing Mix (a mixture of fine peat moss, fine perlite, and vermiculite) soil, with each seed in its own individual tray cell.

1.6 Average predictive comparisons

Average Predictive Comparisons can increase the interpretability of variables in complex (e.g., multilevel and interactive) models (Gelman & Pardoe, 2007). The average predictive comparison for an input variable, υ, is:

$$APC = \widehat{\Delta}\upsilon = \left(\frac{\sum_{i=1}^{n} \sum_{k=1}^{K} \sum_{s=1}^{S} [\sum_{j\epsilon(k)} \omega(\upsilon_i, \upsilon_j)] E(y|\upsilon^k, \nu_i, \theta^s) - E(y|\upsilon_i, \nu_i, \theta^s)}{S \sum_{i=1}^{n} \sum_{k=1}^{K} [\sum_{j\epsilon(k)} \omega(\upsilon_i, \upsilon_j)]} \right)^{\frac{1}{2}} \quad (1)$$

Where n is the number of observations in the data, θ is the model with s independent sets of parameter draws (Bayesian iterations), y is the response variable, υ is the input variable of interest with k levels, and ν is a vector of all the other variables (i.e., all the variables except υ). Average predictive comparisons are distinct from partial derivatives, since APCs do not collapse $\upsilon^2 - \upsilon^1$ to zero, but retain the unit differences found in the data.

Because our study uses a balanced, full factorial design, we can ignore the weighting function, $\omega(\upsilon_i, \upsilon_j)$, which is the likelihood that υ transitions from υ_i to υ_j when $\nu = \nu_i$.

We also calculated the standard error of the average predictive comparison according to:

$$S.E.(\widehat{\Delta}_\upsilon) = \frac{1}{2\widehat{\Delta}_\upsilon} \left(\frac{1}{S-1} \sum_{s=1}^{S} [(\widehat{\Delta}_\upsilon^s)^2 - (\widehat{\Delta}_\upsilon)^2]^2 \right)^{\frac{1}{2}} \quad (2)$$

For a worked example see: http://htmlpreview.github.io/?https://github.com/hneyster/germination$_s$tan/blob/master/APC.html.

Average predictive comparisons complement model results to better interpret our findings. For example, in Figure 2 (main text), the estimates for germination rate are due to inconsistent, idiosyncratic effects that are not generalizable but appear because of a combination of the APC method and these particular results: APCs take the sum of squared differences (see equation above) and shows that seeds stratified at 60 days rather than 30 days had plus or minus 43% probability of germinating (i.e., they were either more likely or less likely to germinate—the model results in Figure 3 show that these positive and negative differences are just random and balance each other out).

References

Andrews, C. J., & Burrows, V. D. (1974). Increasing winter survival of dormant seeds by a treatment inducing secondary dormancy. *Can J. Plant Sci, 54*, 565–571.

Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and evolution of dormancy and germination.* San Diego.

Bush, T., Ogle, D., St. John, L., Stannard, M., & Jensen, K. (2012). *Plant guide for orchardgrass (dactylis glomerata)* (tech. rep.). USDA-Natural Resources Conservation Service, Aberdeen Plant Materials Center. Aberdeen, Idaho 83210.

Casal, J. J., & Sánchez, R. A. (1998). Phytochromes and seed germination. *Seed Science Research, 8*, 317–329.

Colautti, R. I., & MacIsaac, H. J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions, 10*(2), 135–141.

Defelice, M. S. (2001). Shepherd's-purse, capsella bursa-pastoris (l.) medic. 1. *Weed technology, 15*(4), 892–895.

Ellis, R. H., Hong, T. D., & Roberts, E. H. (1986). Quantal response of seed germination in brachiaria humidicola, echinochloa turnerana, eragrostis tef and panicum maximum to photon dose for the low energy reaction and the high irradiance reaction. *Journal of experimental botany, 37*(179), 742–753.

Gelman, A., & Pardoe, I. (2007). Average predictive comparisons for models with nonlinearity, interactions, and variance components. *Sociological Methodology, 37*(1), 23–51.

Holm, L. G., Pancho, J. V., Herberger, J. P., & Plucknett, D. L. (1979). *A geographical atlas of world weeds.* John Wiley; Sons.

Holm, L. G., Plucknett, D. L., Pancho, J. V., & Herberger, J. P. (1977). *The world's worst weeds.* East-West Center Book.

IUCN (International Union for Conservation of Nature). (2008). *About invasive species* (tech. rep.). Invasive Species Specialist Group, IUCN. Gland, Switzerland.

Knobloch, F. (1996). *The culture of wilderness: Agriculture as colonization in the american west.* University of North Carolina Press.

Moser, L., & Buxton, M., D. Casler. (1996). Cool-season forage grasses. agronomy monograph no. 34. *American Society of Agronomy, Crop Science Society of America, Soil Science Society of America*, 503–534.

Munro, D., Steer, J., & Linklater, W. (2019). On allegations of invasive species denialism. *Conservation Biology, 33*(4), 797–802.

Ogle, D., John, L. S., Stannard, M., & Holzworth, L. (2011). *Conservation plant materials for the intermountain west* (tech. rep.) [Technical Note 24.]. USDA-Natural Resources Conservation Service. Boise, ID.

Pons, T. L. (1991). Induction of dark-dormancy in seeds: Its importance for the seed bank in the soil. *Functional Ecology, 5*, 669–675.

Popay, A. I., & Roberts, E. H. (1970). Factors involved in the dormancy and germination of capsella bursa-pastoris (l.) medik. and senecio vulgaris l. *The Journal of Ecology*, 103–122.

Probert, R. J., Smith, R. D., & Birch, P. (1986). Germination response to light and alternating temperatures in european populations of dactylis glomerata l. *New Phytologist*, *99*, 305–316.

Richardson, D. M., Pyšek, P., & Carlton, J. T. (2011). A compendium of essential concepts and terminology in invasion ecology. *Fifty years of invasion ecology: the legacy of Charles Elton*, 409–420.

Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and distributions*, *6*(2), 93–107.

Roberts, E. H., Murdoch, A. J., & Ellis, R. H. (1987). The interaction of environmental factors on seed dormancy. *British crop protection conference weeds*, *7C*(1), 687–694.

Samuelsen, A. B. (2000). The traditional uses, chemical constituents and biological activities of plantago major l. a review. *Journal of Ethnopharmacology*, *71*(1), 1–21.

Tester, M., & Morris, C. (1987). The penetration of light through soil. *Plant, Cell, and Environment*, *10*, 281–286.

USDA, N. (2010). *The plants database.* National Plant Data Center.

USDA Agricultural Research Service. (1971). *Common weeds of the united states.* Dover Publications, Inc.

Westrich, L. (1989). *California herbal remedies.* Gulf Publishing.

2 Raw Data Plots

Below we provide plots of the raw data. In the main text we show model estimates because they provide best estimates that account for much of the variance and pseudoreplication in the data (as the model integrates over seed family, population, and species).
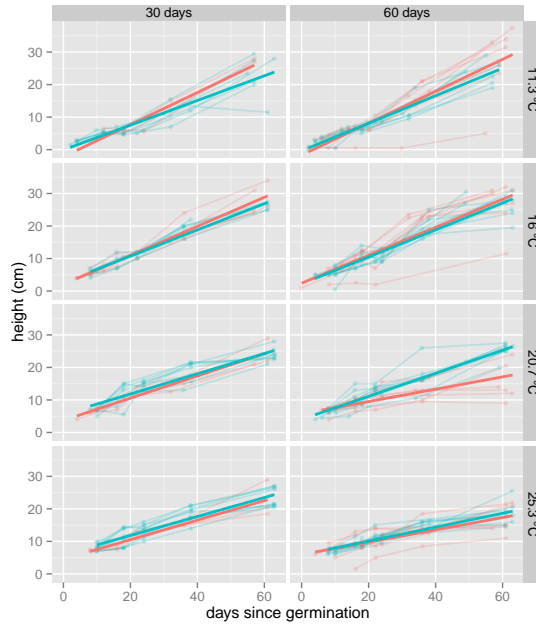
(a) CAPBUR
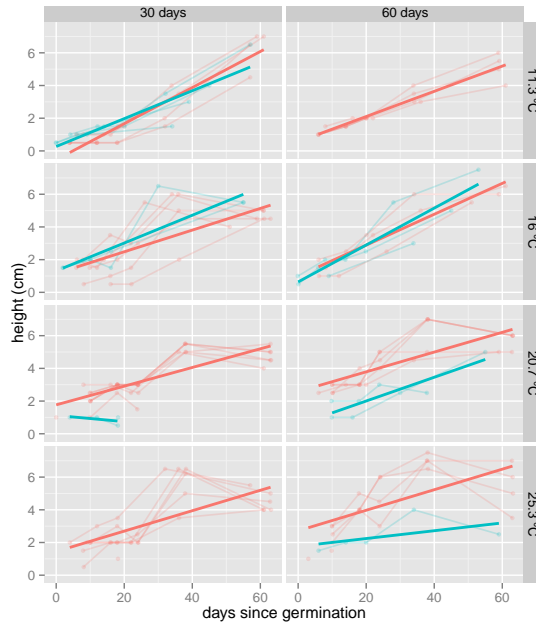
(b) CHEMAJ

(c) DACGLO

(d) PLALAN

(e) PLAMAJ

(f) RUMCRI

(g) TAROFF
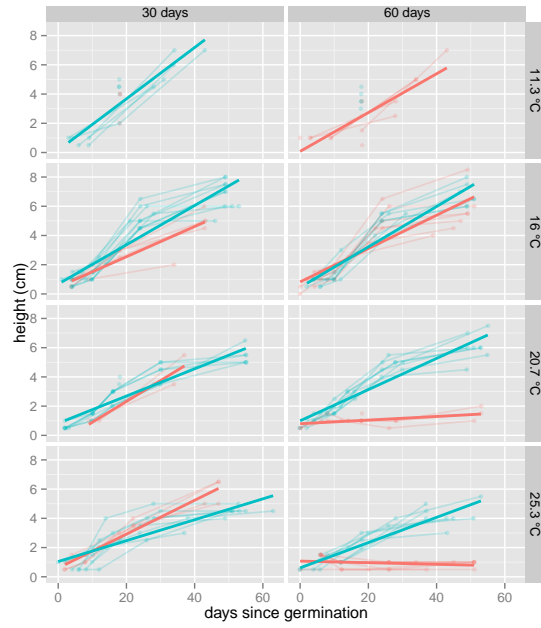
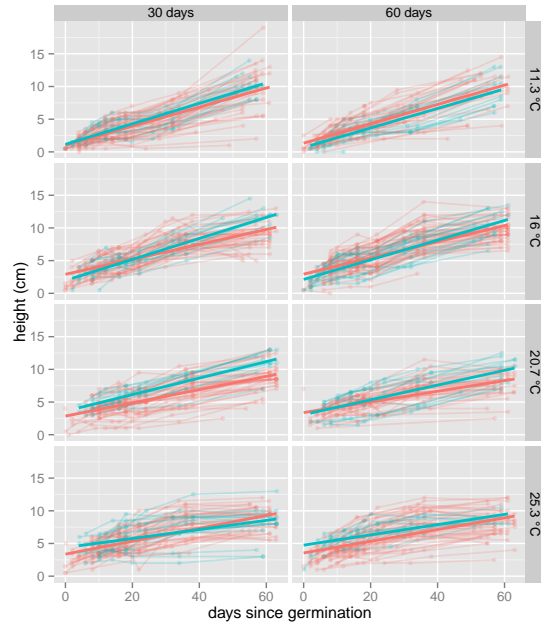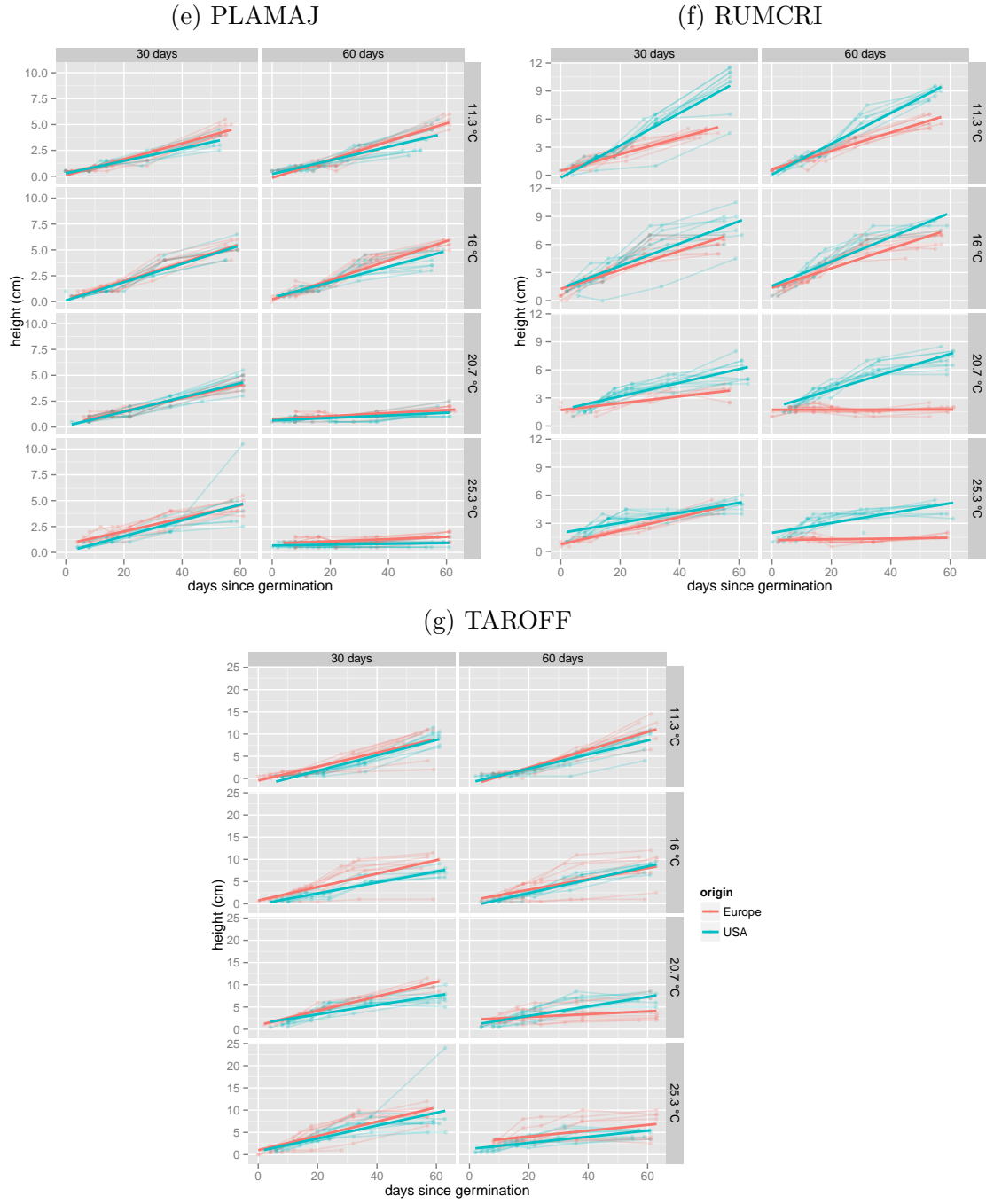Figure S1: Height vs. age by stratification and temperature, which shows that growth rate was approximately linear. Pink represents European plants, while blue represents those from North America

8
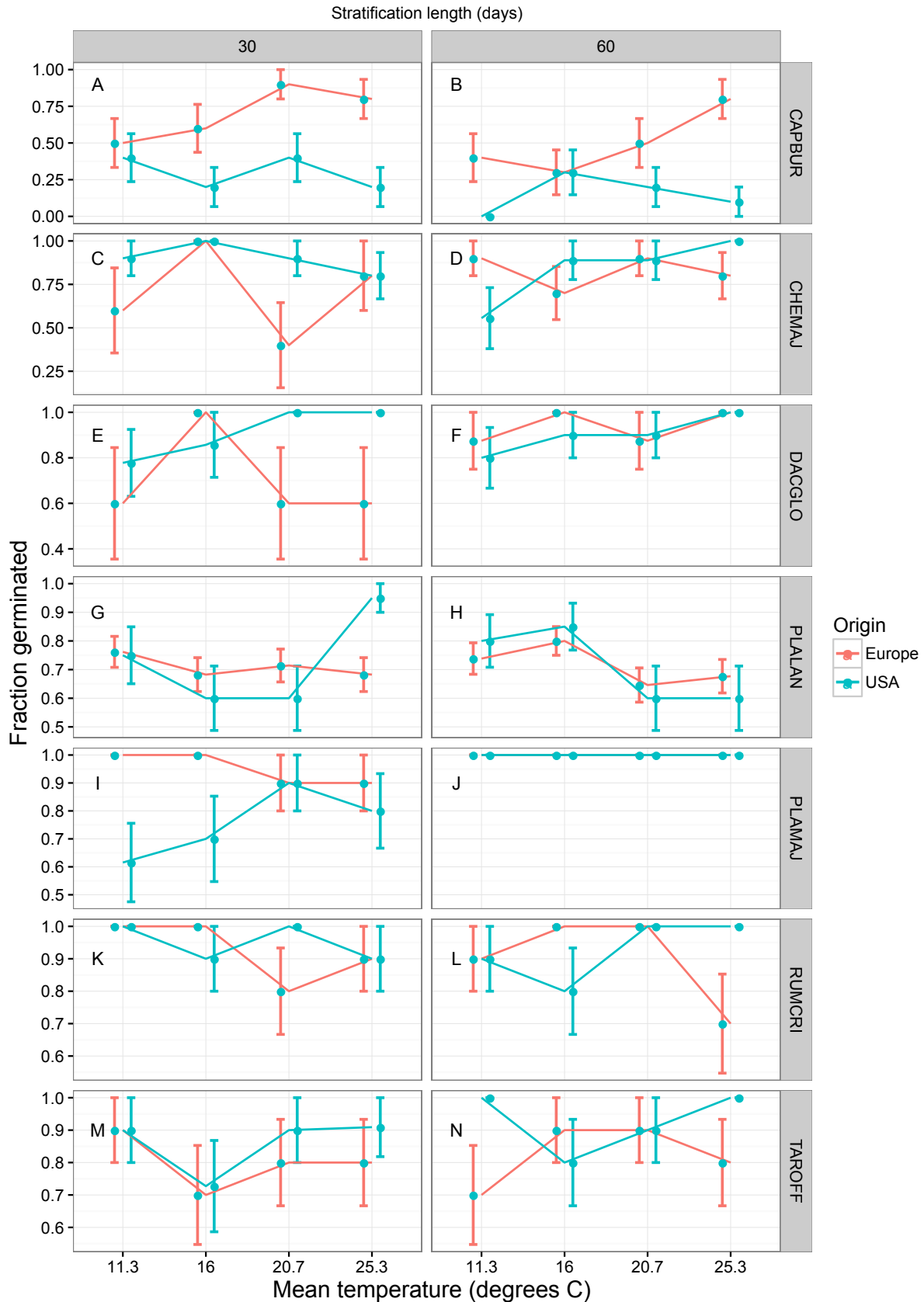
Figure S2: Unmodeled germination rate by population origin, and across stratification length and temperature treatments for each species (mean +/- standard error). Note the variable y-axis scale.

9

Figure S3: Unmodeled germination timing by population origin, and across stratification length and temperature treatments for each species (mean +/- standard error). Note the variable y-axis scale.

Figure S4: Unmodeled growth rate by population origin, and across stratification length and temperature treatments for each species (mean +/- standard error). Note the variable y-axis scale.
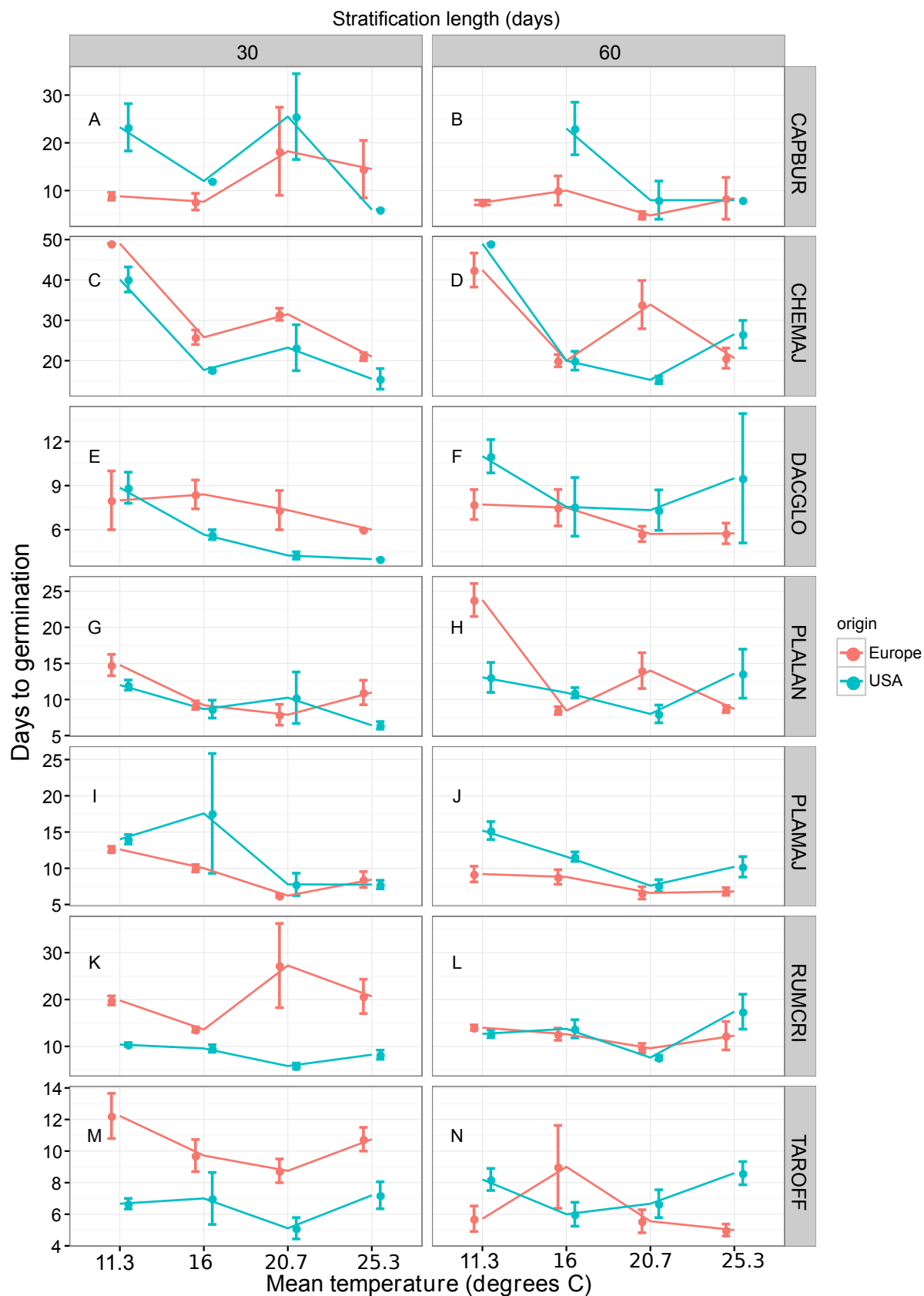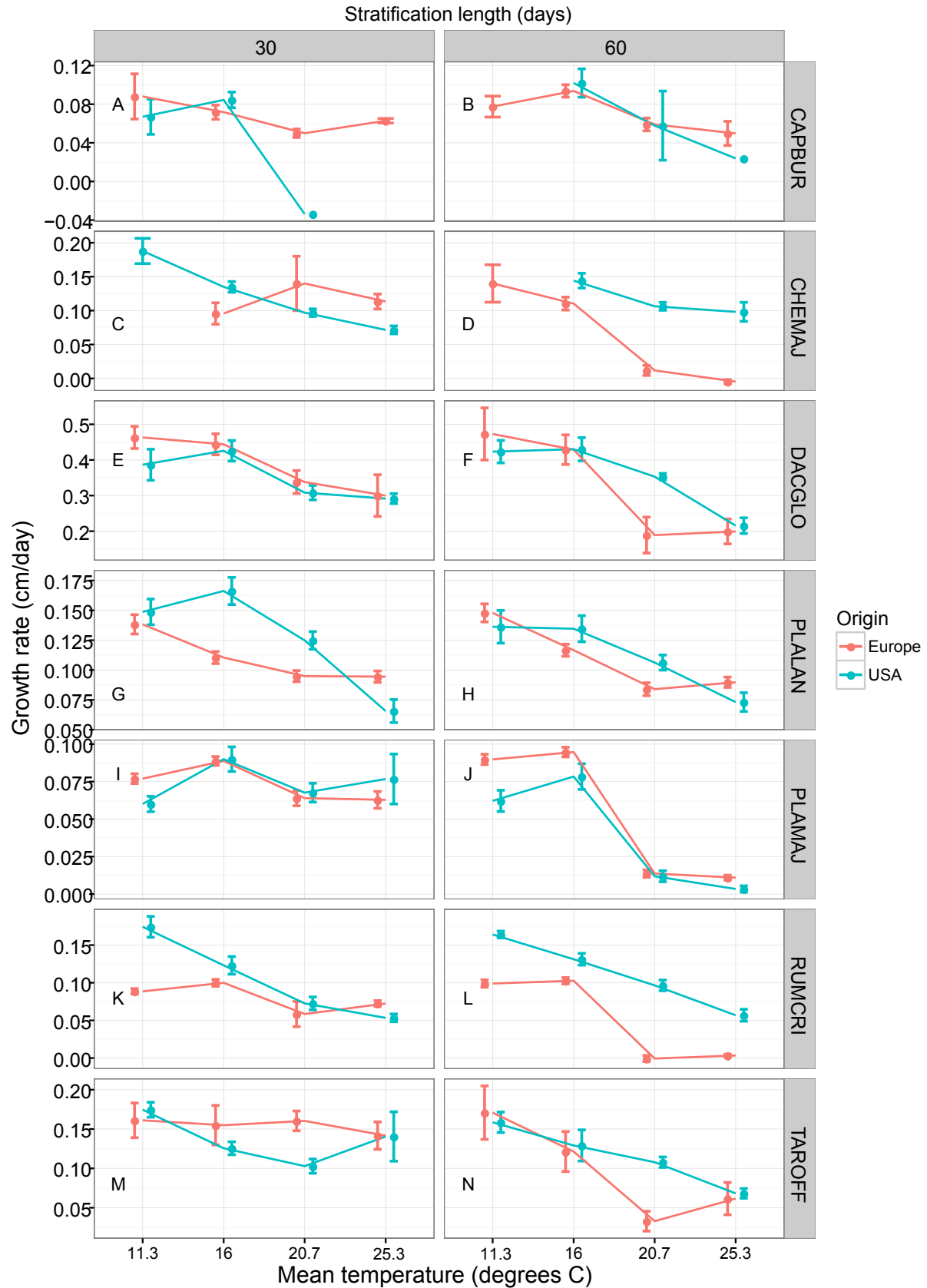
3 Model Results

3.1 Coefficient tables

Table S2: Summary of multilevel model of germination rate. Output is in logit(fraction germinated)

	mean	sd	2.5%	50%	97.5%	Rhat
(Intercept)	1.82	0.48	0.93	1.80	2.79	1.00
origin	-0.10	0.72	-1.50	-0.09	1.33	1.00
strat	0.17	0.68	-1.11	0.14	1.65	1.00
temp1	0.20	0.54	-0.78	0.17	1.31	1.00
temp2	-0.10	0.45	-0.95	-0.11	0.82	1.00
temp3	-0.18	0.43	-1.03	-0.18	0.68	1.00
origin × strat	-0.01	0.84	-1.63	-0.03	1.72	1.00
origin × temp1	-0.41	0.71	-1.78	-0.42	0.97	1.00
origin × temp2	0.76	0.85	-0.82	0.74	2.50	1.00
origin × temp3	0.88	0.75	-0.54	0.87	2.36	1.00
strat × temp1	0.61	0.73	-0.85	0.60	2.05	1.00
strat × temp2	0.45	0.71	-0.86	0.41	1.98	1.00
strat × temp3	0.59	0.76	-0.82	0.55	2.22	1.00
origin × strat × temp1	0.53	1.09	-1.59	0.52	2.72	1.00
origin × strat × temp2	0.11	1.00	-1.82	0.11	2.11	1.00
origin × strat × temp3	0.80	1.36	-1.65	0.73	3.63	1.00

Table S3: Summary of multilevel model of germination timing. Output is in log(days)

	mean	sd	2.5%	50%	97.5%	Rhat
(Intercept)	2.75	0.25	2.28	2.75	3.26	1.00
origin	-0.15	0.17	-0.48	-0.15	0.21	1.00
strat	-0.11	0.19	-0.50	-0.10	0.22	1.00
temp1	-0.37	0.11	-0.58	-0.37	-0.13	1.00
temp2	-0.47	0.16	-0.75	-0.47	-0.14	1.00
temp3	-0.36	0.14	-0.62	-0.37	-0.07	1.00
origin × strat	0.16	0.20	-0.22	0.16	0.56	1.00
origin × temp1	0.06	0.18	-0.29	0.06	0.41	1.00
origin × temp2	0.05	0.24	-0.43	0.05	0.49	1.00
origin × temp3	-0.21	0.19	-0.59	-0.21	0.16	1.00
strat × temp1	0.05	0.19	-0.32	0.05	0.46	1.00
strat × temp2	-0.09	0.20	-0.48	-0.09	0.33	1.00
strat × temp3	-0.23	0.16	-0.53	-0.24	0.11	1.00
origin × strat × temp1	0.01	0.24	-0.49	0.01	0.48	1.00
origin × strat × temp2	-0.07	0.34	-0.77	-0.06	0.59	1.00
origin × strat × temp3	0.57	0.26	0.05	0.58	1.06	1.00

Table S4: Summary of multilevel model of growth rate. Output is in cm/day

	mean	sd	2.5%	50%	97.5%	Rhat
(Intercept)	0.16	0.05	0.07	0.16	0.25	1.00
origin	0.01	0.01	-0.02	0.01	0.03	1.00
strat	0.01	0.01	-0.01	0.01	0.02	1.00
temp1	-0.02	0.01	-0.04	-0.02	0.01	1.00
temp2	-0.04	0.01	-0.07	-0.04	-0.02	1.00
temp3	-0.04	0.02	-0.08	-0.04	-0.01	1.00
origin × strat	-0.01	0.01	-0.03	-0.01	0.02	1.00
origin × temp1	0.01	0.01	-0.02	0.01	0.03	1.00
origin × temp2	-0.02	0.02	-0.05	-0.02	0.01	1.00
origin × temp3	-0.03	0.02	-0.06	-0.03	0.01	1.00
strat × temp1	-0.00	0.01	-0.03	-0.00	0.02	1.00
strat × temp2	-0.06	0.02	-0.11	-0.06	-0.03	1.00
strat × temp3	-0.06	0.02	-0.11	-0.06	-0.02	1.00
origin × strat × temp1	0.01	0.02	-0.03	0.01	0.04	1.00
origin × strat × temp2	0.07	0.03	0.02	0.07	0.13	1.00
origin × strat × temp3	0.04	0.02	-0.01	0.04	0.09	1.00

3.2 Inter-population variability
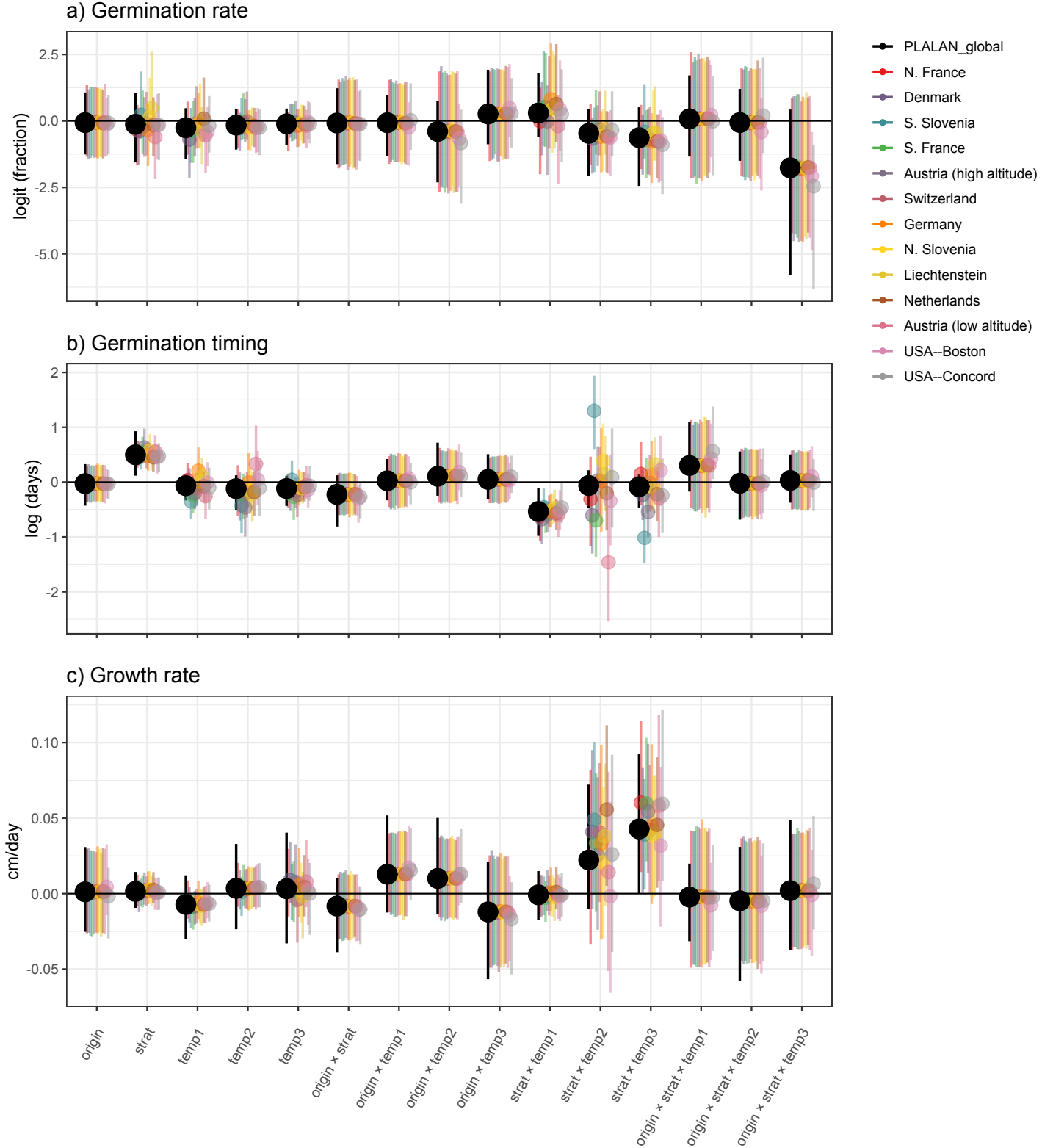
Figure S5: Germination rate, germination timing, and growth rate multilevel model coefficients with 95% credible intervals for *Plantago lanceolata*, showing main random effect for the species and random effects of each population. Zero represents the global mean fixed effect across all species. All values are relative to the intercept coefficients (see Tables S5, S6, S7). The reference level for temperature is 18/8°C, while temp1 = 22.67/12.67°C, temp2 = 27.33/17.33°C, and temp3 = 32/22°C. Thirty days is the reference level for stratification (strat).

Table S5: Germination rate model intercepts across all species (global intercept), across all populations of PLALAN (PLALAN intercept), and for each population of PLALAN. Note that all PLALAN intercepts are relative to the global intercept. Output is in logit(fraction germinated)

	mean	sd	2.5%	50%	97.5%	Rhat
global intercept	1.82	0.48	0.93	1.80	2.79	1.00
PLALAN intercept	-0.15	0.39	-1.08	-0.06	0.54	1.00
N. France PLALAN intercept	-1.41	0.69	-2.79	-1.40	-0.11	1.00
Denmark PLALAN intercept	-1.03	0.63	-2.30	-1.02	0.20	1.00
S. Slovenia PLALAN intercept	1.06	0.69	-0.19	1.03	2.46	1.00
S. France PLALAN intercept	0.99	0.78	-0.47	0.95	2.57	1.00
Austria (high altitude) PLALAN intercept	0.98	0.74	-0.41	0.96	2.49	1.00
Switzerland PLALAN intercept	1.10	0.78	-0.34	1.07	2.70	1.00
Germany PLALAN intercept	-2.11	0.79	-3.65	-2.12	-0.55	1.00
N. Slovenia PLALAN intercept	0.61	0.70	-0.72	0.59	2.06	1.00
Liechtenstein PLALAN intercept	-0.07	0.67	-1.38	-0.07	1.23	1.00
The Netherlands PLALAN intercept	0.05	0.66	-1.25	0.04	1.34	1.00
Austria (low altitude) PLALAN intercept	-1.33	0.68	-2.68	-1.30	-0.05	1.00
USA–Boston PLALAN intercept	-0.26	0.85	-1.94	-0.25	1.38	1.00
USA–Concord PLALAN intercept	0.02	0.86	-1.66	0.01	1.79	1.00

Table S6: Germination timing model intercepts across all species (global intercept), across all populations of PLALAN (PLALAN intercept), and for each population of PLALAN. Note that all PLALAN intercepts are relative to the global intercept. Output is in log(days to germination)

	mean	sd	2.5%	50%	97.5%	Rhat
global intercept	2.75	0.25	2.28	2.75	3.26	1.00
PLALAN intercept	-0.10	0.25	-0.61	-0.10	0.39	1.00
N. France PLALAN intercept	-0.04	0.13	-0.31	-0.03	0.21	1.00
Denmark PLALAN intercept	0.13	0.13	-0.08	0.12	0.42	1.00
S. Slovenia PLALAN intercept	0.10	0.12	-0.11	0.09	0.37	1.00
S. France PLALAN intercept	-0.01	0.12	-0.25	-0.01	0.22	1.00
Austria (high altitude) PLALAN intercept	0.14	0.14	-0.08	0.12	0.44	1.00
Switzerland PLALAN intercept	-0.05	0.12	-0.31	-0.04	0.18	1.00
Germany PLALAN intercept	0.07	0.14	-0.20	0.05	0.37	1.00
N. Slovenia PLALAN intercept	-0.15	0.14	-0.47	-0.14	0.07	1.00
Liechtenstein PLALAN intercept	-0.19	0.15	-0.52	-0.18	0.05	1.00
The Netherlands PLALAN intercept	0.04	0.12	-0.19	0.03	0.30	1.00
Austria (low altitude) PLALAN intercept	-0.01	0.12	-0.26	-0.01	0.23	1.00
USA–Boston PLALAN intercept	-0.00	0.15	-0.30	-0.00	0.30	1.00
USA–Concord PLALAN intercept	-0.02	0.15	-0.33	-0.01	0.27	1.00

Table S7: Growth rate model intercepts across all species (global intercept), across all populations of PLALAN (PLALAN intercept), and for each population of PLALAN. Note that all PLALAN intercepts are relative to the global intercept. Output is in cm/dat

	mean	sd	2.5%	50%	97.5%	Rhat
global intercept	0.16	0.05	0.07	0.16	0.25	1.00
PLALAN intercept	-0.03	0.05	-0.12	-0.03	0.07	1.00
N. France PLALAN intercept	-0.01	0.01	-0.03	-0.01	0.02	1.00
Denmark PLALAN intercept	-0.03	0.01	-0.05	-0.03	-0.01	1.00
S. Slovenia PLALAN intercept	0.01	0.01	-0.01	0.01	0.03	1.00
S. France PLALAN intercept	0.00	0.01	-0.02	0.00	0.02	1.00
Austria (high altitude) PLALAN intercept	-0.01	0.01	-0.02	-0.01	0.01	1.00
Switzerland PLALAN intercept	-0.01	0.01	-0.02	-0.01	0.01	1.00
Germany PLALAN intercept	0.02	0.01	-0.01	0.02	0.04	1.00
N. Slovenia PLALAN intercept	0.02	0.01	0.00	0.02	0.04	1.00
Liechtenstein PLALAN intercept	0.01	0.01	-0.01	0.01	0.03	1.00
The Netherlands PLALAN intercept	0.00	0.01	-0.02	0.00	0.02	1.00
Austria (low altitude) PLALAN intercept	-0.02	0.01	-0.04	-0.02	0.00	1.00
USA–Boston PLALAN intercept	0.01	0.01	-0.02	0.01	0.04	1.00
USA–Concord PLALAN intercept	-0.01	0.01	-0.04	-0.01	0.02	1.00