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Research

Testing the association of relative growth rate and adaptation to climate across natural ecotypes of *Arabidopsis*

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Summary

- Ecophysiologists have reported a range of relationships, including intrinsic trade-offs across and within species between plant relative growth rate in high resource conditions (RGR) vs adaptation to tolerate cold or arid climates, arising from trait-based mechanisms. Few studies have considered ecotypes within a species, in which the lack of a trade-off would contribute to a wide species range and resilience to climate change.
- For 15 ecotypes of *Arabidopsis thaliana* in a common garden we tested for associations between RGR vs adaptation to cold or dry native climates and assessed hypotheses for its mediation by 15 functional traits.
- Ecotypes native to warmer, drier climates had higher leaf density, leaf mass per area, root mass fraction, nitrogen per leaf area and carbon isotope ratio, and lower osmotic potential at full turgor. Relative growth rate was statistically independent of the climate of the ecotype native range and of individual functional traits.
- The decoupling of RGR and cold or drought adaptation in *Arabidopsis* is consistent with multiple stress resistance and avoidance mechanisms for ecotypic climate adaptation and would contribute to the species' wide geographic range and resilience as the climate changes.

Introduction

Climate change is increasingly impacting plant populations and species across ecological contexts worldwide, necessitating general principles for prediction (Cook *et al.*, 2004; Cayan *et al.*, 2008; AghaKouchak *et al.*, 2014; Diffenbaugh *et al.*, 2015; Fournier-Level *et al.*, 2016). One strong constraint often hypothesized to influence the physiology and distribution of species is an intrinsic trade-off within or among species between growth and adaptation to stress, including to cold or arid climates (Grime, 1974, 1977 Bloom *et al.*, 1985; Smith & Huston, 1989; Sartori *et al.*, 2019). However, a fully integrative view of resilience in the face of cold or drought suggests multiple possible relationships with growth rate (synthesized with nomenclature defined in Fig. 1). Thus, resilience can be achieved by tolerance (i.e. withstanding the impact of stress), and/or recovery (Levitt, 1980; Hodgson *et al.*, 2015; Volaire, 2018), and tolerance in turn can be achieved through resistance (i.e. a maintenance of function during the stress), and/or avoidance (i.e. by confining growth to favorable warm and wet periods). Species or genotypes may achieve stress resistance through mechanisms associated with low growth rate or independent of growth rate, whereas stress avoidance is typically associated with high growth rate (Berger *et al.*, 2016). Thus, whether a trade-off between growth and adaptation to cold or drought arises will depend on the type(s) of tolerance possessed. Indeed, our compilation of studies shows diverse 'growth-stress tolerance relationships' (GSTRs; synthesis in Supporting Information Table S1). Of 23 previous studies, 13 supported a trade-off between growth rate and adaptation to cold or dry climates (Polley *et al.*, 2002; Griffith *et al.*, 2007; Atwell *et al.*, 2010; Darychuk *et al.*, 2012; Koehler *et al.*, 2012; Molina-Montenegro *et al.*, 2012; Lopez-Iglesias *et al.*, 2014; Kaproth & Cavender-Bares, 2016; Vasseur *et al.*, 2018; Leites *et al.*, 2019; Lubbe & Henry, 2019; Sartori *et al.*, 2019; Ramirez-Valiente *et al.*, 2020), five found them to be *positively coordinated* (McKay *et al.*, 2003; Kenney *et al.*, 2014; Ramirez-Valiente *et al.*, 2017, 2020; Vasseur *et al.*, 2018) and 10 found them to be *decoupled* (Fernández & Reynolds, 2000; Polley *et al.*, 2002; Sack, 2004; Sanchez-Gomez *et al.*, 2006, 2008; Atwell *et al.*, 2010; Mukherjee *et al.*, 2011; Bristiel *et al.*, 2018; Leites *et al.*, 2019; Jung *et al.*, 2020), with some showing support for multiple relationships. Yet, understanding GSTRs and their potential limitation on growth remains urgent (Hilty *et al.*, 2021) as they can profoundly affect species range width and niche specialization, ecotype differentiation and turnover across a climate gradient, and how these properties may shift with climate change (synthesis in A1). A trade-off between maximum relative growth rate (RGR) and cold or drought tolerance across ecotypes would in principle result in a smaller species climatic range and greater climatic sensitivity relative to positive coordination or decoupling (A1).

We tested for an association between RGR and adaptation to cold and drought for ecotypes of a particularly significant genetic and ecological model, an annual herb with a very wide climatic distribution, *Arabidopsis thaliana* (hereafter Arabidopsis). Further, we quantified phenotypic traits related to cold and drought tolerance, and their putative roles in constraining RGR. Trait-based mechanisms, including biomass allocation and leaf economics, have been proposed to drive associations of growth rate and adaptation to cold and aridity (Table 1). Some have hypothesized that trade-offs would arise with growth rate if plants achieve cold or drought resistance by allocating to higher root mass fraction (RMF) and higher leaf dry mass per unit area (LMA), which by reducing allocation to photosynthetic leaf surface would result in lower RGR (Fig. 1; Smith & Huston, 1989; Sterck *et al.*, 2011; Poorter *et al.*, 2012). Some have also hypothesized that trade-offs would arise from 'leaf economics spectrum' (LES) trait relationships that frequently appear within and across plant species (Wright *et al.*, 2004), namely between traits associated with either slow or rapid carbon and nutrient acquisition. Previous work in Arabidopsis suggested that high LMA (the product of leaf thickness and density; Witkowski & Lamont, 1991) and low leaf nitrogen per area and per mass (N_{area} and N_{mass}) contribute to slow RGR and are associated with cold or dry habitats (Sartori *et al.*, 2019). Additionally, plants may achieve cold or drought resistance through other adaptations with potential costs to RGR, such as a high osmotic concentration, which reduces wilting point, contributing to drought resistance and potentially chilling or freezing resistance (Parker, 1963; Gonzalez-Zurdo *et al.*, 2016), while potentially restricting maximum stomatal opening, and thereby rates of gas exchange and RGR (Henry *et al.*, 2019). By contrast, a positive coordination between RGR and adaptation to cold or dry climates may arise for 'stress-avoiding' annual species with short times to flowering, or in deciduous species, via traits that enable a high RGR when resources are available (Maximov, 1931; Grubb, 1998; McKay *et al.*, 2003; Kikuzawa *et al.*, 2013; Kenney *et al.*, 2014; Vitasse *et al.*, 2014; Gonzalez-Zurdo *et al.*, 2016). Finally, RGR and stress resistance may be decoupled if species or ecotypes achieve tolerance via traits that confer resistance without necessarily reducing RGR, such as a smaller leaf size or greater leaf thickness, or if the set of species or ecotypes considered include both resistant and avoidant types (Wanner & Junttila, 1999; Fernández & Reynolds, 2000; Sack, 2004;

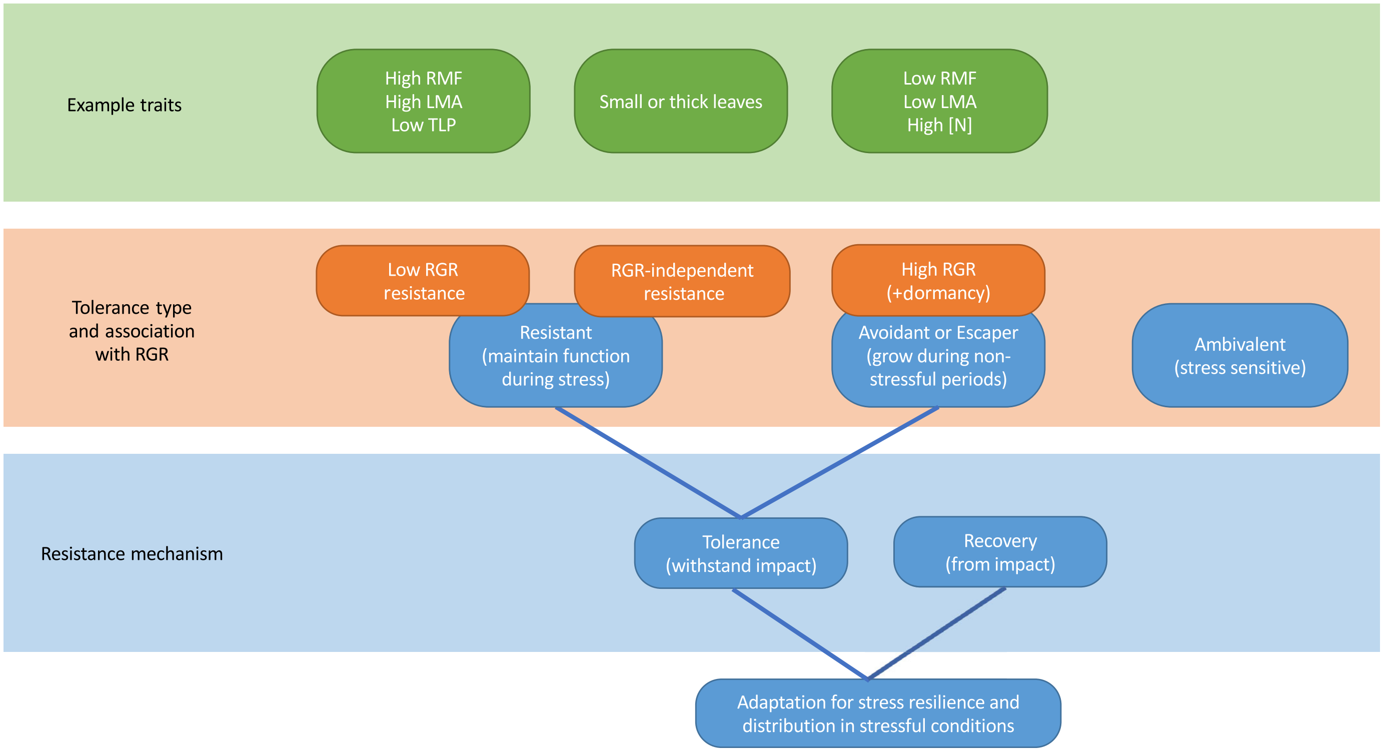

Fig. 1 Schematic depicting mechanisms for achieving drought tolerance, with nomenclature defined, slightly revised after Levitt (1980), Hodgson *et al.* (2015) and Volaire (2018). Adaptation for stress resilience and distribution in stressful conditions (at the bottom of schematic) is based on the plant's ability to tolerate (i.e. withstand) or recover from the impact of stress. Tolerance can be achieved by either resisting stress, that is maintaining function, or avoiding (or escaping) the stress by surviving the stressful period and/or growing during nonstressful periods; nontolerant plants are 'stress-ambivalent' or sensitive. Resistance can be achieved through mechanisms that are theoretically mechanistically linked to low relative growth rate (RGR), with example traits listed such as high root mass fraction (RMF), high leaf mass per area (LMA) and lower turgor loss point (π_{TLP}; Table 1). Resistance can also be achieved through mechanisms independent of RGR, with example traits listed such as small or thick leaves (Table 1). Avoidance can be achieved through traits that contribute to high RGR (and often coupled to dormancy during stress), with example traits listed such as low RMF, low LMA or high foliar nitrogen concentration (see Table 1 for references for the roles of functional traits).

Sanchez-Gomez *et al.*, 2006, 2008; Yadav, 2010; Mukherjee *et al.*, 2011; Wright *et al.*, 2017; Bristiel *et al.*, 2018; Ding *et al.*, 2019; Jung *et al.*, 2020; Table 1).

Ecotypes of model species

Arabidopsis provides an ideal platform for testing GSTRs and their basis in functional traits. Important questions were raised by the data of two previous studies that showed weak trends of RGR with native climate across large sets of ecotypes (strongest r^2-values of RGR relationships with climate variables were 0.07–0.12; Atwell *et al.*, 2010; Vasseur *et al.*, 2018; Fig. S1). One study suggested, based on linear correlations across 451 ecotypes, a trade-off between RGR and cold adaptation, and positive coordination with dry climate adaptation (Vasseur *et al.*, 2018; Fig. S1; Table S1). Our reanalysis of those data showed that a U-shaped relationship for RGR vs mean annual temperature (MAT) was statistically selected over a linear model, as high RGR ecotypes were native to both cold and very warm climates (Fig. S1a,b; Tables S1, S2). By contrast, our analysis of data for 60 ecotypes (Atwell *et al.*, 2010) indicated that RGR was positively coordinated with cold adaptation, and decoupled from adaptation to aridity (Fig. S1c,d; Tables S1, S2). In those previous studies, RGR was estimated as rosette area expansion, which can differ from mass-based RGR, which better represents whole-plant function (Inman-Narahari *et al.*, 2014; Falster *et al.*, 2018), and functional trait-based mechanisms were not quantified.

We focused on 15 Arabidopsis ecotypes representing populations native to a wide range of climates across Europe and Asia (Weigel & Mott, 2009), and grown in a glasshouse common garden (Table 2). We tested for an association across ecotypes between RGR and adaptation to cold and/or aridity. We also hypothesized that across ecotypes, RGR would be related to flowering time. Additionally, we tested whether RGR was related to 15 traits that we hypothesized to be associated with climate and/or flowering times, given they have been described as contributing to resistance or avoidance of cold or dry climates in the published literature for diverse species (hypothesized trait–climate relationships reviewed in Table 1), including reproductive traits such as seed mass and flowering times; biomass allocation traits such as RMF and reproductive mass fraction (ReproMF); relative growth rate components including specific leaf area (SLA; the inverse of LMA), leaf mass fraction (LMF), leaf area ratio (LAR) and unit leaf rate (ULR), where RGR = ULR × LAR, and LAR = SLA × LMF (Evans, 1972; Hunt, 1990; Lambers *et al.*, 1998); leaf morphological traits such as leaf size, thickness and density; and leaf composition and biochemistry traits such as Chl per area (Chl/area), carbon isotope ratio (δ^{13}C), N_{area}, N_{mass} and the leaf osmotic potential at full turgor (π_o), the main biophysical determinant of wilting point (i.e. turgor loss point; Bartlett *et al.*, 2012a,b; Fletcher *et al.*, 2018; Griffin-Nolan *et al.*, 2019). We also tested for correlations among leaf economics traits, that is that LMA, N_{area} and Chl/area would be positively correlated and LMA and N_{mass} would be negatively correlated (Wright *et al.*, 2004), and whether this variation was associated with RGR. We further considered how relationships of RGR with adaptation to cold and/or dry climates would potentially influence the native range of the species, and its responses to climate warming and aridification.

Materials and Methods

Growth conditions

We grew 15 *A. thaliana* (L.) Heynh. ecotypes in a climate-controlled glasshouse common garden at the University of California, Los Angeles. Following previous studies of adaptation in Arabidopsis (Vasseur *et al.*, 2018; Sartori *et al.*, 2019), rather than imposing drought or cold treatments we on focused plants in a warm, well-watered common garden to test for adaptation of phenotypic traits and growth to native cold and dry climates.

Seeds were acquired from The Arabidopsis Information Resource (TAIR; Huala *et al.*, 2001), from the collection of the 1001 Genotypes Project (Weigel & Mott, 2009; Alonso-Blanco *et al.*, 2016), in which ecotypes represent populations from which multiple individuals were sampled to represent phenotypic and genetic diversity. Fifteen ecotypes were selected for variation in aridity index and temperature across seven Arabidopsis 'origin' groups (Table 2; Trabucco & Zomer, 2019). Seeds for each ecotype were bulked up in the experimental glasshouse and sown in lawns on soil (18.75% washed plaster sand, 18.75% sandy loam, 37.5% peat moss, 12.5% perlite, 12.5% coarse vermiculite) in pots (8 cm wide, 8 cm long, 9 cm deep), placed in a cold room (4°C) on 13 April 2016, and moved to glasshouse benches on 18 April 2016. Seedlings of each ecotype were then transplanted two to a pot ($n = 17$ pots per ecotype; 8 cm long, 12.3 cm wide, 6 cm deep) at the five true-leaf stage on 3–9 May 2016, randomized across two benches, thinned to one seedling per pot 2 wk later, and staked after 3 wk. Ten plants of each ecotype were selected randomly for harvesting on 21–23 June 2016. Glasshouse mean temperature was 22.9°C (minimum 20.6°C, maximum 26.3°C), mean humidity was 48% (21–64%) and mean irradiance at plant level from 09:00 to 16:00 h was 193 µmol photons $\mathrm{m^{-2}\,s^{-1}}$ (26–533 µmol photons $\mathrm{m^{-2}\,s^{-1}}$; HOBO Micro Station with Smart Sensors, Onset, Bourne, MA, USA), with no significant differences in these variables between the two benches (comparisons at mid-day for 17 d; *t*-tests, $P > 0.07$ for all tests). Plants were watered two or three times per week with fertilized water (250 ppm of Peters Professional water-soluble fertilizer; ICL Fertilizer, Dublin, OH, USA; N 20%, P 20%, K 20%, B 0.0125%, Cu 0.0125%, Fe 0.05%, Mn 0.025%, Mo 0.005%, Zn 0.025%).

Plant harvesting and biomass and trait measurements

For each ecotype, mean seed mass was determined by dividing the mass of 50 seeds by 50. On 3 May 2016, at the time of transplanting the five-leaf seedlings from lawns to pots, for the 12 of 15 ecotypes with seedlings abundantly available, an initial harvest was conducted of 25 seedlings per ecotype, enabling determination of mean initial seedling dry mass. At maturity (i.e. between 69 and 71 d), after siliques formed and just began to brown, 10 individuals per ecotype were randomly selected for measurement

Table 1 Traits measured for 15 ecotypes of *Arabidopsis thaliana* grown experimentally, with hypotheses and rationales for expected correlations (positive, +; negative, –; not applicable, na) with cold or arid native climates based on stress tolerance or stress avoidance (i.e. rapid growth in the period with favorable climate), and for association with high relative growth rate (RGR) or leaf mass per unit area (LMA).

Trait	Units	Hypothesized correlations with climatic temperature or moisture given adaptation for resistance to cold or aridity or to stress avoidance			Hypothesized relationship to RGR	Hypothesized relationship to LMA	Rationales for hypothesized linkages of traits with climate adaptation and RGR	Min., Avg., Max.	ANOVA for differences among ecotypes; df14
		Adaptation to cold resistance	Adaptation to aridity resistance	Stress avoidance					
Reproductive									
Seed mass	g	+	+	+	+		Seedlings from large seeds tend to have greater cold or drought tolerance due to greater germination rates and greater ability to allocate to rapid root growth before stress; within a given species, seedlings from larger seeds tend to have higher RGR during establishment[1,2]	$1. \times 56 \times 10^{-5}$, $\mathbf{2.14 \times 10^{-5}}$, 3.86×10^{-5}	na[†]
Flowering time (FT10/FT16)	d	na	na	+/–	–		Early flowering genotypes can complete their life cycle earlier during favorable periods and better avoid stress[3] and tend to have higher RGR.[3–7]. Yet, early flowering can also increase the chance to experience late frost[8]	60.5, **73.1**, 94.3/ 38.0, **57.0**, 89.3	na[‡]
Relative growth rate components									
Unit leaf rate (ULR)	$g\ m^{-2}\ d^{-1}$	na	na	+	+		As a component of RGR, a higher ULR would confer faster growth during favorable periods and avoidance of stress periods[9,10]	4.93, **14.9**, 35.9	324, 0.65***
Leaf mass fraction (LMF)	$g\ g^{-1}$	–	–	+	+		A lower LMF would confer greater resistance of cold or drought as it represents a greater mass allocation to resource capture and storage below ground.[11] As a component of RGR, a higher LMF would confer faster growth during favorable periods and avoidance of stress periods[9,10]	0.11, **0.42**, 0.76	0.301, 0.79***
Leaf mass per area (LMA; inverse of specific leaf area, SLA)	$g\ m^{-2}$	+	+ ✓	–	–		A higher LMA would confer greater resistance of cold or drought, corresponding to lower evaporative surface, greater water mass per leaf area and greater leaf mechanical protection.[11,12] Specific leaf area, the inverse of LMA, is a component of RGR, and thus would confer faster growth during favorable periods and avoidance of stress periods[9,10,13]	18.7, **30.5**, 59.2	761, 0.76***

Table 1 (Continued)

Trait	Units	Hypothesized correlations with climatic temperature or moisture given adaptation for resistance to cold or aridity or to stress avoidance			Hypothesized relationship to RGR	Hypothesized relationship to LMA	Rationales for hypothesized linkages of traits with climate adaptation and RGR	Min., Avg., Max.	ANOVA for differences among ecotypes; df14
		Adaptation to cold resistance	Adaptation to aridity resistance	Stress avoidance					
Other biomass allocation									
Reproductive mass fraction (ReproMF)	$g\ g^{-1}$	na	na	+	–		Allocating more strongly to reproduction can potentially reflect earlier diversion of resources to complete the life cycle earlier during favorable periods and better avoid stress.[2] However, allocating more strongly to reproductive tissues may entail a cost to photosynthetic tissues contributing to RGR[14]	0.07, **0.51**, 0.85	0.38, 0.81***
Root mass fraction (RMF)	$g\ g^{-1}$	+	+ ✓	–	–		A higher root mass fraction would confer resistance of cold or drought via mass allocation to resource capture and storage below ground.[11] However, allocating more strongly to root tissues may entail a cost to photosynthetic tissues and thus RGR[15]	0.02, **0.08**, 0.17	0.012, 0.50***
Other leaf morphological traits									
Leaf area (LA)	cm^2	–	–	+/–	+/– ✓		Smaller leaf area results in a thinner boundary layer, and leaves more closely coupled with air temperature, thus avoiding overheating and chilling damage, and achieving higher photosynthetic rates and water use efficiency under dry conditions, as well as higher photosynthetic rates under moist warm conditions, promoting more rapid growth in favorable climates.[16] Alternatively, larger leaves can increase LAR and thus increase RGR[17]	0.62, **2.57**, 5.88	11.35, 0.69***
Leaf thickness (LT)	mm	+	+	+/–	+/– ✓	+ ✓	Greater leaf thickness would contribute to higher LMA, and its benefits for stress resistance at the cost of RGR, as described above[12,18] but may also correspond to greater nitrogen and Chl concentrations per area, contributing to higher RGR	0.06, **0.16**, 0.10	0.0049,0.77***
Leaf density (LD)	$mg\ mm^{-3}$	+	+ ✓	–	–	+ ✓	A denser leaf would contribute to higher LMA, and its benefits for stress resistance at the cost of RGR, as described above[12,18–20]	0.21, **0.30**, 0.43	0.022, 0.36*

Table 1 (Continued)

Trait	Units	Hypothesized correlations with climatic temperature or moisture given adaptation for resistance to cold or aridity or to stress avoidance			Hypothesized relationship to RGR	Hypothesized relationship to LMA	Rationales for hypothesized linkages of traits with climate adaptation and RGR	Min., Avg., Max.	ANOVA for differences among ecotypes; df14
		Adaptation to cold resistance	Adaptation to aridity resistance	Stress avoidance					
Leaf composition and biochemistry									
Chl per area (Chl/area)	SPAD	na	na	+	+ ✓	a+, m− ✓	A higher Chl concentration may contribute to greater light harvesting and to faster RGR under favorable climates[21]	19.49, **30.10**, 45.87	307, 0.80***
Osmotic potential at full turgor (π_o)	MPa	–	− ✓	+	+		A more negative osmotic potential may confer chilling resistance and results in a more negative turgor loss point, directly conferring drought resistance.[22,23] However, the higher turgor pressure in hydrated leaves may restrict stomatal opening[24], with a negative consequence for RGR under favorable conditions	−1.02, **−0.89**, −0.79	0.063, 0.33***,§
Carbon isotope ratio ($\delta^{13}C$)	‰	na	− ✓	+	–		A more negative carbon isotope ratio indicates greater discrimination against ^{13}C, suggesting more of their stomata are open, which would lead to increased photosynthesis and faster growth.[25,26] Increases in $\delta^{13}C$ are related to increases in water use efficiency, aiding survival in arid climates[27]	−33.53, **−31.65**, −29.01	7.28, 0.82***
Leaf nitrogen per mass and area (N_{mass} and N_{area})	mg g^{-1}	na	na	+ ✓	+	a+, m− ✓	Higher leaf nitrogen content would enable faster photosynthetic rates per leaf mass or area[19], potentially allowing for rapid growth during periods of water availability	34.11, **47.18**, 62.16/0.89, **1.36**, 2.31	347, 0.58***/na†

Hypotheses were compiled based on the published literature for comparisons of populations within Arabidopsis or other species, or for comparisons across species. A check mark indicates that our study found support for the hypothesis across the Arabidopsis ecotypes. Measured trait variation is presented (minimum, average (bold) and maximum mean ecotype values) along with the mean squares from the results of analysis of variance (ANOVA) testing for variation among ecotypes, with the proportion of variance explained by differences among ecotypes (the remainder being explained within ecotypes), and significance levels for each trait for specified degrees of freedom (df): ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; na, not applicable (also see Supporting Information Table S3). Trait names in bold type were hypothesized to be intercorrelated as part of the leaf economics spectrum (LES); a or m preceding the predicted correlation indicates the expectation according to the mass- or area-based LES respectively.

References: 1. Gomez (2004); 2. Turnbull *et al*. (2012); 3. Griffith & Watson (2005); 4. Kazan & Lyons (2016); 5. Kenney *et al*. (2014); 6. Meyre *et al*. (2001); 7. Vasseur *et al*. (2018); 8. Bigler & Bugmann (2018); 9. Hunt (1990); 10. Hunt & Cornelissen (1997); 11. Sack *et al*. (2003); 12. Gonzalez-Zurdo *et al*. (2016); 13. Poorter *et al*. (2009); 14. Sartori *et al*. (2019); 15. Poorter *et al*. (2012); 16. Wright *et al*. (2017); 17. Conesa *et al*. (2019); 18. Niinemets (2001); 19. Wright *et al*. (2004); 20. Evans (1972); 21. Chaturvedi *et al*. (2011); 22. Bartlett *et al*. (2012b); 23. Parker (1963); 24. Henry *et al*. (2019); 25. Farquhar *et al*. (1989); 26. McKay *et al*. (2003); 27. Wang *et al*. (2016).

[†] Single mean values were determined for ecotypes: seed mass as mean of 50 seeds per ecotype, and N_{area} was determined as mean $N_{mass} \times$ LMA.

[‡] Flowering times taken as single mean values in Alonso-Blanco *et al*. (2016).

[§] df = 7 for osmotic potential values.

Table 2 Ecotypes of *Arabidopsis thaliana* grown experimentally, in order of ascending aridity index (with a larger value indicating greater humidity of the native climate), indicating substantial variation in leaf traits.

Ecotype	Origin group	LMA ($g\,m^{-2}$)	FT10 (d)	FT16 (d)	Aridity index	MAP ($mm\,yr^{-1}$)	MAT (°C)
CS76789*	Relict	46.4 (6.3)	62.8	47.3	0.138	281	22.7
CS76649	Relict	24.7 (1.9)	63.0	41.0	0.259	497	14.1
CS76532*	Asia	56.1 (5.4)	78.5	61.8	0.305	493	14.2
CS77002*	Italy/Balkan/Caucasus	27.8 (1.1)	77.3	78.8	0.671	773	12.2
CS76778 (Col-0)	Germany	22.6 (1.9)	70.5	38.0	0.721	1023	13.1
CS76748	Central Europe	19.2 (1.5)	64.0	71.8	0.863	806	10.9
CS76897*	Germany	20.9 (1.7)	68.0	48.8	0.789	640	7.32
CS76379*	Asia	59.2 (5.1)	79.3	73.0	0.598	705	2.70
CS78855	Central Europe	26.1 (3.5)	94.3	58.8	0.829	648	6.52
CS78888*	Admixed	30.0 (3.9)	71.8	51.0	0.923	838	9.54
CS76498	Germany	21.5 (3.2)	71.0	44.5	0.986	801	8.35
CS78916*	Admixed	28.6 (1.8)	81.3	89.3	1.026	810	9.54
CS77170*	Central Europe	35.7 (3.6)	84.3	62.5	0.972	705	4.72
CS76382	Asia	20.1 (2.5)	69.8	51.3	0.639	557	−2.87
CS76623	Western Europe	18.7 (2.2)	60.5	38.0	1.703	1572	10.0

Values of leaf mass per area (LMA) are means with standard error in parentheses. Values of flowering time at 10°C (FT10) and 16°C (FT16) represent mean values for each ecotype (Alonso-Blanco *et al*., 2016). Other climate variables include mean annual precipitation (MAP) and mean annual temperature (MAT). An asterisk indicates the ecotypes that were measured for osmotic potential at full turgor (π_o).

of five plants for biomass harvesting and five for leaf osmotic potential at full turgor. For biomass harvesting, plants were separated into photosynthetic and senescent leaves, inflorescence, basal stem and roots, which were washed of soil, and all parts were dried at 70°C for at least 72 h before weighing for dry mass (XS205; Mettler, Toledo, OH, USA). Before drying, three randomly selected rosette leaves were traced for leaf area measurement using IMAGEJ (v.1.46r), and measured for leaf thickness (Fowler Digital Calipers, Chicago, IL, USA) and Chl concentration per leaf area, using a SPAD meter (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan), which provides measurements in SPAD units that correlate with total ($a+b$) Chl per leaf area (Chl/area; Uddling *et al*., 2007). Root, leaf (rosette and inflorescence leaves) and other (basal stem, flower and fruit) mass fractions were calculated by dividing mass values by the plant total dry mass.

Relative growth rate (RGR; $g\,g^{-1}\,d^{-1}$) was calculated as

$$\mathrm{RGR} = \frac{\log_e\left(\frac{M_f}{M_0}\right)}{\Delta t} \qquad \text{Eqn1}$$

where M_f is the dry mass at final harvest (g), M_0 is the initial mass (g) and Δt is time (days between initial and final harvest). Absolute growth rate (AGR; $g\,d^{-1}$) was calculated as

$$\mathrm{AGR} = \frac{M_f - M_0}{\Delta t} \qquad \text{Eqn2}$$

Relative growth rate and AGR were determined in two ways: first, using M_0 for the seed mass for all 15 ecotypes (Kitajima, 1994), and second, using the initial harvest seedling mass (five-leaf stage) for the 12/15 ecotypes for which sufficient seedlings were available for initial seedling harvest. As the AGR and RGR values resulting from these two calculation approaches were highly correlated across ecotypes ($r = 0.99$ and 0.89 respectively; $P < 0.001$; $n = 12$), the more complete dataset for RGR using seed mass is presented, with the other dataset in Table S3. M_f was also highly correlated with AGR ($r = 0.99$; $P < 0.001$), so AGR is presented in the text, with M_f data available in Table S3. Leaf mass fraction (LMF; $g\,g^{-1}$) was calculated as leaf mass (g) divided by M_f; leaf dry mass per unit area (LMA; $g\,m^{-2}$) as leaf mass (g) divided by leaf area (m^2); leaf area ratio ($m^2\,g^{-1}$) as leaf area divided by M_f; and the unit leaf rate (ULR; $g\,m^{-2}\,d^{-1}$) as RGR divided by LAR. Leaf density ($g\,m^{-3}$) was calculated as LMA divided by leaf thickness (mm; Evans, 1972). Root mass fraction (RMF; $g\,g^{-1}$) was calculated as root mass divided by M_f, and base and reproductive (including inflorescence stems, fruits and flowers) mass fractions were calculated as the mass of those parts divided by M_f. Three dry leaves from each of three to five plants per genotype were ground and analyzed for nitrogen per leaf mass (N_{mass}; $mg\,g^{-1}$) and carbon isotope ratio ($\delta^{13}C$) using dual isotope analysis with an Elemental Analyzer interfaced to a mass spectrometer (Center for Stable Isotope Biogeochemistry at the University of California, Berkeley, CA, USA). Nitrogen concentration per leaf area (N_{area}; $g\,m^{-2}$) was determined as N_{mass} multiplied by LMA.

The osmotic potential at full turgor (π_o) was estimated using the osmometer method during the harvest period (Bartlett *et al*., 2012a) on a randomly selected subset of eight ecotypes (Table 2). Whole plants were rehydrated overnight in plastic bags, and two leaf disks per each of five plants per ecotype were punched, immediately submerged in liquid nitrogen, and placed into the osmometer to be measured for osmolality (Vapro 5520 and 5600 vapor pressure osmometer; Wescor, Logan, UT, USA), which was then converted to π_o (Bartlett *et al.*, 2012a; Table S3). Notably, the osmometer measurement of π_o is correlated with the turgor loss point (π_{TLP}) with lower values corresponding to greater drought resistance across woody species (Bartlett *et al.*, 2012a,b), herb species (Griffin-Nolan *et al.*, 2019; Sun *et al.*, 2020) and cultivars/populations of given species (Banks & Hirons, 2019; Mart *et al.*, 2016; Rosas *et al.*, 2019).

Climate and flowering time data

We followed previous studies in testing relationships of RGR and traits with the climate of ecotypes' sampling sites (Weigel & Mott, 2009) based on climate variables modeled at 1 km^2 resolution using the WorldClim database (Zanne *et al.*, 2014; Fletcher *et al.*, 2018; Vasseur *et al.*, 2018; Sartori *et al.*, 2019; Baird *et al.*, 2021), and in assuming that the modeled mean native climate for ecotypes was representative, despite uncertainty in the correspondence of the single point sampled to the mean climate and microclimate of the ecotype's range (Hancock *et al.*, 2011; Lasky *et al.*, 2012; Alonso-Blanco *et al.*, 2016; Mojica *et al.*, 2016; Vasseur *et al.*, 2018; Sartori *et al.*, 2019; Lorts & Lasky, 2020). Coordinates for each ecotype were obtained from the 1001 Genomes Consortium 2016 (https://1001genomes.org/accessions.html). Mean annual temperature and precipitation data were downloaded from WorldClim v.2.1 Global Climate Data (BioClim; http://www.worldclim.org/bioclim; Fick & Hijmans, 2017), and monthly temperature and precipitation variables were downloaded from the WorldClim historical climate database (Harris *et al.*, 2014; Fick & Hijmans, 2017). Additionally, aridity index data were obtained from the Consultative Group for International Agriculture Research (CGIAR) Consortium for Spatial Information (CSI) database v.2 (Trabucco & Zomer, 2019; Table S3). Climate information was extracted for the coordinates for each ecotype using ArcMap (v.10.0). Growing season mean climate variables, including an estimate of the potential maximum length of the growing season, were calculated for the months with ≥ 4°C mean temperature and precipitation ≥ 2× mean temperature (Lasky *et al.*, 2012). Notably, Arabidopsis genotypes are diverse in life history, and some genotypes overwinter as rosettes and flower in early spring, while others germinate and complete their lifecycles in spring and/or early summer (Mitchell-Olds & Schmitt, 2006). Life history information is not available for the genotypes in this study, and thus growing season variables are subject to a level of uncertainty as for previous studies (Lasky *et al.*, 2012, 2014). While we include growing season mean climate variables in the Results, we feature mean annual climate variables in plots, as they are subject to fewer assumptions, and were strongly correlated with growing season means (see the Results section). We obtained flowering time (days until first open flower) for each of the 15 ecotypes from the 1001 Genomes Consortium, based on growth experiments at a constant temperature of 10°C or 16°C after an initial cold treatment (FT10 and FT16, respectively; Alonso-Blanco *et al.*, 2016; Table S3).

Statistical analyses

Trait variation across ecotypes and quantification of the relative trait variation within and among ecotypes was tested using analyses of variance (*aov* function in the STATS package) in the R Statistics environment (R v.3.5.1).

To test relationships across ecotypes for growth, trait and climate variables, a linear mixed effects model accounting for kinship was implemented using the *lmekin* function in the COXME package in R. Kinship matrices were obtained from the 1001 Genomes Project data release v.3.1 (Weigel & Mott, 2009). To test both linear and nonlinear (power law) associations of traits with climate, we analyzed both untransformed and log-transformed data. Before log-transformation, variables with negative units (i.e. π_o and $\delta^{13}C$) were multiplied by -1; for mean annual temperature, which included negative and positive values, a constant equal to the lowest mean value for an ecotype +1 was added for all ecotypes such that the lowest value was 1. We present in the main text the most significant relationship for untransformed or log-transformed data, and all results in Tables S4–S7.

We analyzed the causal importance of components of RGR (i.e. ULR, LAR, LMF and SLA). As tests of the correlations of RGR with its individual components can be influenced by their covariation and do not resolve direct causal influences on RGR (John *et al.*, 2017), we applied a causal partitioning analysis (Buckley & Diaz-Espejo, 2015; John *et al.*, 2017). In this approach, considering y, a function of a number (N) of variables x_j (i.e. $y = f(x_1, x_2, \ldots, x_N)$), an infinitesimal change, dy, is expressed as the sum of infinitesimal changes caused by each variable: $\mathrm{d}y = \frac{\partial y}{\partial x_1}\mathrm{d}x_1 + \frac{\partial y}{\partial x_2}\mathrm{d}x_2 + \cdots + \frac{\partial y}{\partial x_N}\mathrm{d}x_N = \sum_{j=1}^{N}\frac{\partial y}{\partial x_j}\mathrm{d}x_j$. Integrating this expression between the data for ecotypes A and B ($y_A = f(x_{1A}, x_{2A}, \ldots)$ and $y_B = f(x_{1B}, x_{2B}, \ldots)$) produces a sum of finite terms representing the contributions of each variable x_j to the difference between y_A and y_B: $\int_A^B \mathrm{d}y = y_A - y_B = \int_A^B \frac{\partial y}{\partial x_1}\mathrm{d}x_1 + \int_A^B \frac{\partial y}{\partial x_2}\mathrm{d}x_2 + \cdots + \int_A^B \frac{\partial y}{\partial x_N}\mathrm{d}x_N = \sum_{j=1}^{N}\left[\int_A^B \frac{\partial y}{\partial x_j}\mathrm{d}x_j\right]$. Expressing each term in this sum as a percentage of the total change in y then gives the % contribution of each x variable ($C(x_k)$) to the change in y:

$$\%\ \text{causal contribution of } x_k \text{ to the difference in } y \text{ between ecoytpes} \equiv C(x_k) = 100 \cdot \frac{\int_A^B \frac{\partial y}{\partial x_k}\mathrm{d}x_k}{\sum_{j=1}^{N}\left[\int_A^B \frac{\partial y}{\partial x_j}\mathrm{d}x_j\right]} = 100 \cdot \frac{\int_A^B \frac{\partial y}{\partial x_k}\mathrm{d}x_k}{y_A - y_B} \qquad \text{Eqn3}$$

The % causal contribution of any given variable x_k to y can be positive or negative, and by definition they add up to 100%. Applying to this analysis for $y = \mathrm{RGR} = \mathrm{LAR} \times \mathrm{ULR} = \mathrm{SLA} \times \mathrm{LMF} \times \mathrm{ULR}$, a higher positive % causal contribution for a factor indicates that, on average, that factor plays a stronger causal role in determining higher RGR across ecotypes. A negative % causal contribution indicates that for an ecotype with higher RGR, that causative factor varies in the direction that would cause a lower RGR, and that this effect is generally overcome by the other causative factors. We applied this partitioning for every possible pairwise comparison between ecotypes (for 15 ecotypes, this gives $15!/[13! \times 2!] = 105$ pairwise comparisons), considering the ecotypes with lesser and higher RGR as ecotypes A and B respectively. We applied this analysis hierarchically: first we partitioned differences in $\log_e(\text{RGR})$ (= y) into contributions from $\log_e(\text{ULR})$ (= x_1) and $\log_e(\text{LAR})$ (= x_2); we then partitioned differences in $\log_e(\text{LAR})$ into contributions from $\log_e(\text{LMF})$ and $\log_e(\text{SLA})$. The partial derivatives in each case were unity (e.g. $\partial\log_e(\text{RGR})/\partial\log_e(\text{ULR}) = 1$), so the % contribution of each variable x_k was calculated as $100(x_{kA}-x_{kB})/(y_A-y_B)$. We then calculated the median value, over all 105 comparisons, for the contribution of each variable (i.e. $C(\log_e[\text{ULR}])$ and $C(\log_e[\text{LAR}])$ for RGR, and $C(\log_e[\text{LMF}])$ and $C(\log_e[\text{SLA}])$ for LAR). Finally, we combined those results into a single set of three values ($C(\log_e[\text{ULR}])$, $C'(\log_e[\text{LMF}])$ and $C'(\log_e[\text{SLA}])$) representing the contributions of ULR, LMF and SLA, respectively, to differences in RGR, by defining $C'(\log_e[\text{LMF}]) = 0.01 \times C(\log_e[\text{LAR}]) \times C(\log_e[\text{LMF}])$ and $C'(\log_e[\text{SLA}]) = 0.01 \times C(\log_e[\text{LAR}]) \times C(\log_e[\text{SLA}])$ so that $C(\log_e[\text{ULR}]) + C'(\log_e[\text{LMF}]) + C'(\log_e[\text{SLA}]) = 100\%$.

Results

Variation in growth functional traits across ecotypes

The 15 Arabidopsis ecotypes native to diverse climates varied substantially in their growth rates (Table 1). AGR varied eightfold from 0.002 to 0.012 $\text{g g}^{-1}\,\text{d}^{-1}$, and RGR 1.3-fold from 0.12 to 0.16 $\text{g g}^{-1}\,\text{d}^{-1}$ (Fig. 2a,b), and AGR and RGR were positively correlated ($r = 0.95$, $P < 0.001$; Tables S4, S5).

The ecotypes varied strongly in all functional traits. They varied seven-fold in both ULR and LMF, and over three-fold in specific SLA (Fig. 2c–e). Ecotypes varied by nearly ten-fold in RMF (from 0.018 to 0.175 g g^{-1}; Fig. 2f) and in leaf area (from 0.62 to 5.88 cm^2), 2.6-fold in leaf thickness (from 0.062 to 0.16 mm), 2-fold in leaf density (from 0.21 to 0.43 mg mm^{-3}) and by −0.23 MPa in π_o (from −0.79 to −1.02 MPa). Two-thirds or more of trait variation arose among rather than within ecotypes for all traits except RMF and π_o, for which half to a third of the variation arose between ecotypes, respectively (Table 1).

Testing the associations of growth with climate variables and flowering time

Across the 15 ecotypes, RGR and AGR were statistically independent of the mean precipitation, aridity and temperature of the native range (Figs 3, S2; Table S4). There was a nonsignificant empirical trend for a positive coordination of higher RGR and lower mean annual temperature ($P = 0.08$; Fig. 3a). Relative growth rate was correlated with published flowering times for the study ecotypes grown at 10°C ($r = 0.64$, $P = 0.007$; Tables S4, S5), which were also statistically independent of the mean precipitation, aridity and temperature of the native range (Table S4).

Absolute growth rate and RGR correlated positively with flowering times published for the study ecotypes grown at 10°C and/or at 16°C ($r = 0.47$–0.63, $P = 0.002$–0.04; Tables S4, S5).

Testing the associations of RGR with its components and other functional traits

Across ecotypes, RGR was not correlated with any single one of its components, ULR, LAR, SLA or LMF (Table S4). Partitioning the causality of RGR by its components across ecotype pairs showed that, while any component could be an important determinant of RGR differences, on average, higher RGR was primarily caused by greater LMF (which contributed, on average across ecotypes, 100.9% of the difference in RGR), with much lesser average contributions from greater ULR (10.5%). The causal effect of SLA was negative (−11.4%; Table 3); thus, ecotypes with higher RGR values tended to have lower SLA values, an influence on average overcome by their higher ULR and especially higher LMF.

Across ecotypes, RGR was coordinated with 3/15 functional traits in the direction hypothesized (Table 1). Ecotypes with higher RGR had larger and thicker leaves with higher Chl concentration per area ($r = 0.46$–0.59, $P = 0.005$–0.5; Fig. 4; Tables S4, S5). Ecotypes with higher RGR had lower carbon isotope ratios ($\delta^{13}\text{C}$; $r = -0.58$, $P = 0.006$; Tables S4, S5). Ecotypes with higher LMF and LAR tended to have greater individual leaf area ($r = 0.64$–0.81, $P \leq 0.001$; Tables S4, S5).

Correlations of traits with climate variables and flowering time

Across the 15 Arabidopsis ecotypes, 5/15 morphological and physiological traits hypothesized to confer drought tolerance were significantly associated with native climate aridity. Thus, LMA, leaf density and RMF were higher, and π_o and $\delta^{13}\text{C}$ were lower for ecotypes with native distributions in lower mean annual precipitation, precipitation of the growing season and/or aridity index ($|r| = 0.48$–0.80, $P < 0.05$; Figs 5a–c,e, 6; Tables S4–S7). Additionally, as expected for adaptation to climatic aridity, ecotypes with lower π_o were native to higher mean annual temperatures ($|r| = 0.62$; $P = 0.03$; Tables S6, S7). None of the traits hypothesized to correlate with cold tolerance based on published relationships for diverse species were associated with native climatic temperature in the expected direction (Table 1). The only supported hypothesis for a trait contribution to stress avoidance (Table 1) was higher leaf nitrogen per area (N_{area}) in species native to greater climatic aridity ($r = 0.48$ with aridity index, $P = 0.03$; Fig. 5d; Tables S4, S5).

Many traits were correlated with published flowering times for the study ecotypes grown at 10°C and/or at 16°C. Leaf area, Chl concentration (Chl/area), LMF, $\delta^{13}\text{C}$ and nitrogen per area were positively correlated, and N_{mass} was negatively correlated with flowering times ($|r| = 0.45$–0.63, $P = 0.002$–0.05; Tables S4, S5).

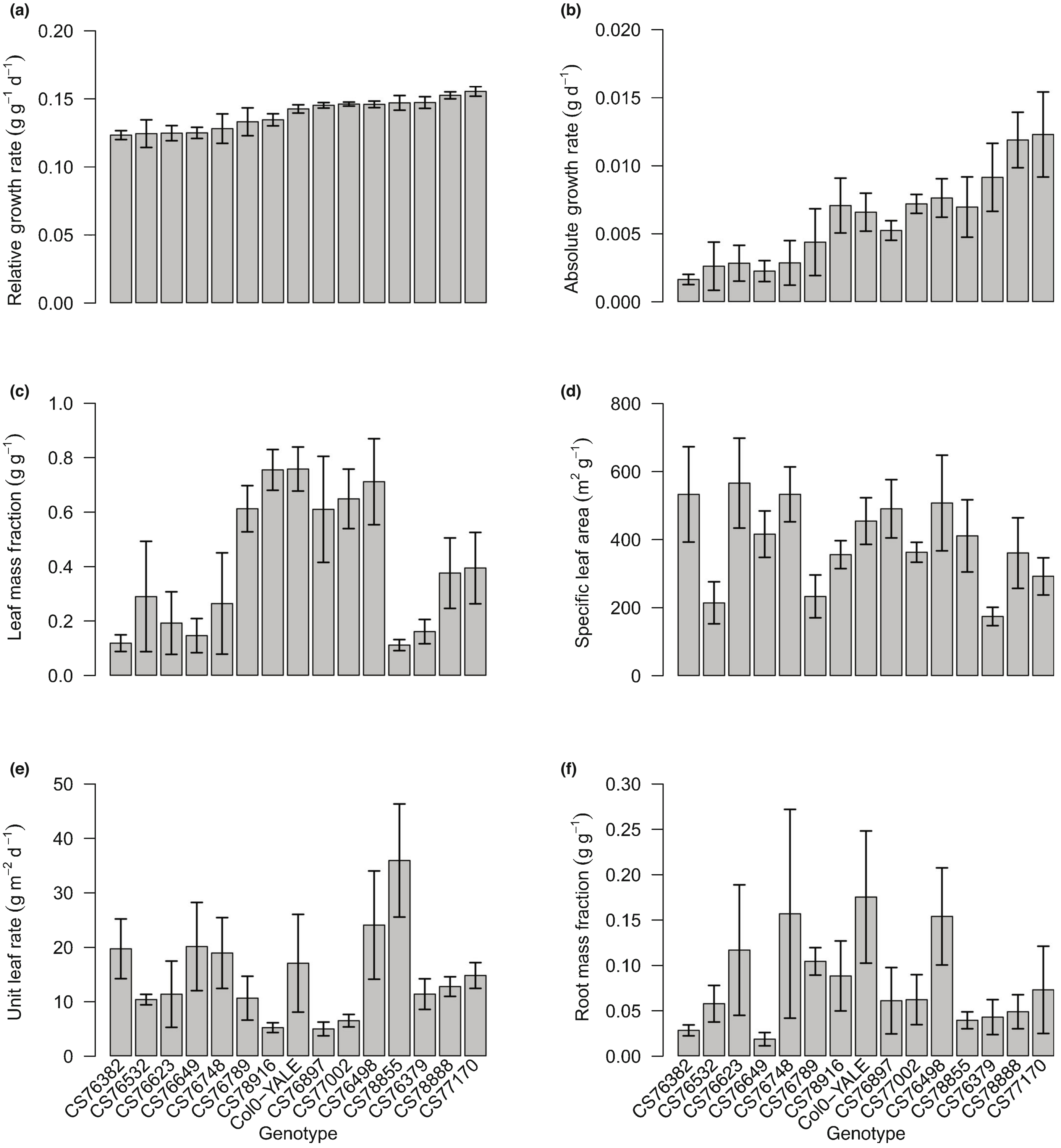

Fig. 2 Variation across 15 Arabidopsis ecotypes grown in a glasshouse common garden in (a) relative growth rate, (b) absolute growth rate, (c) leaf mass fraction, (d) specific leaf area, (e) unit leaf rate and (f) root mass fraction. Ecotypes are ordered from lowest to highest value of relative growth rate. Error bars indicate standard error.

Correlations among leaf economics traits

Across Arabidopsis ecotypes, LES traits were intercorrelated (Tables 1, S4, S5). LMA was positively related to its components, leaf thickness and leaf density, and negatively with nitrogen per leaf mass (N_{mass}; $|r| = 0.47–0.82$, $P \leq 0.04$; Fig. 7; Tables S4, S5). A higher LMA was associated with higher Chl per area and N_{area}, which themselves were correlated ($|r| = 0.78–0.89$,

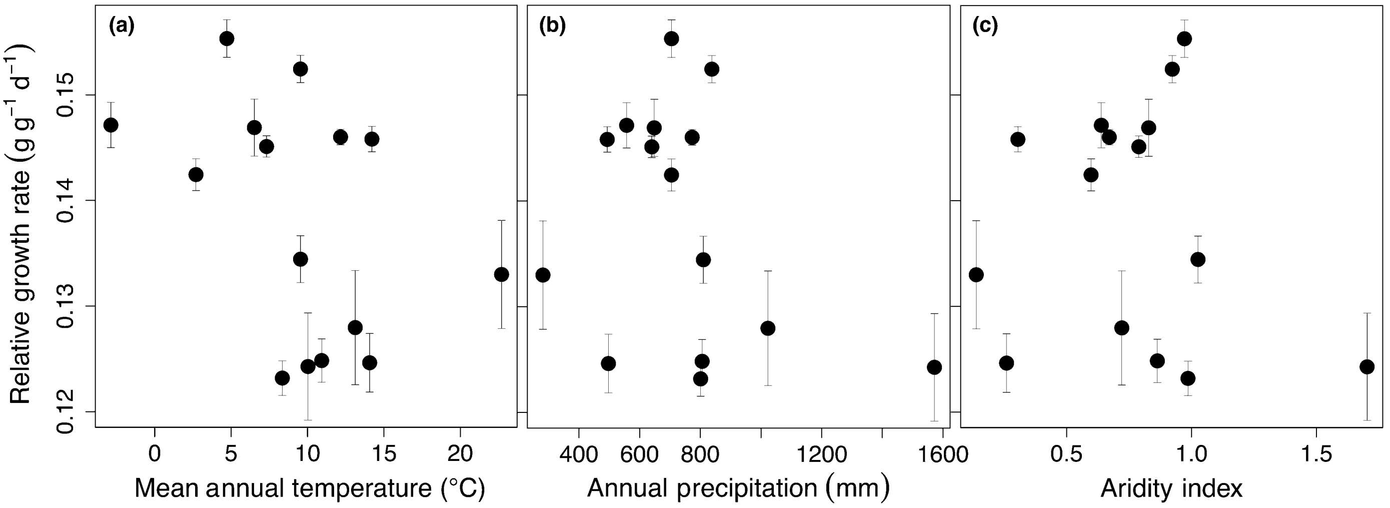

Fig. 3 Relationships of relative growth rate (RGR) with native climate for 15 Arabidopsis ecotypes grown in a glasshouse common garden, that is with (a) mean annual temperature, (b) annual precipitation and (c) aridity index. Error bars indicate standard error.

Table 3 Causal partitioning of log-transformed values of relative growth rate (RGR) into its components, unit leaf rate (ULR) and leaf area ratio (LAR), where LAR can be further partitioned into its components, leaf mass fraction (LMF) and specific leaf area (SLA), to show how much of the observed differences in RGR across *Arabidopsis thaliana* ecotypes are due to differences in each component.

Partitioning	Causal trait	Median % contribution	Interquartile range
RGR into ULR and LAR	ULR	10.5	−734, 800
	LAR	89.5	−700, 834
LAR into LMF and SLA	LMF	112.7	59, 185
	SLA	−12.7	−85, 41
RGR into ULR, LMF, and SLA	ULR	10.5	−734, 800
	LMF	100.9	−573, 1068
	SLA	−11.4	−592, 225

Median values are displayed with the interquartile ranges.

$P < 0.001$; Fig. 8, Tables S4, S5). RGR was positively correlated with leaf thickness and δ^{13}C ($|r| = 0.47$–0.58, $P = 0.006$–0.04; Tables S4, S5).

Discussion

Across the 15 Arabidopsis ecotypes of diverse climatic origin, RGR under high resource conditions was statistically independent of native climatic cold and aridity. Although our study of 15 ecotypes does not preclude weak associations that might emerge across a larger sampling of ecotypes, the lack of support for an RGR–stress tolerance trade-off across a diverse set of ecotypes measured in detail indicates no absolute, intrinsic physiological trait-mediated trade-offs (Smith & Huston, 1989; Sack, 2004; Fine *et al.*, 2006; Sterck *et al.*, 2011). The decoupling of growth from climatic adaptation was consistent with the statistical

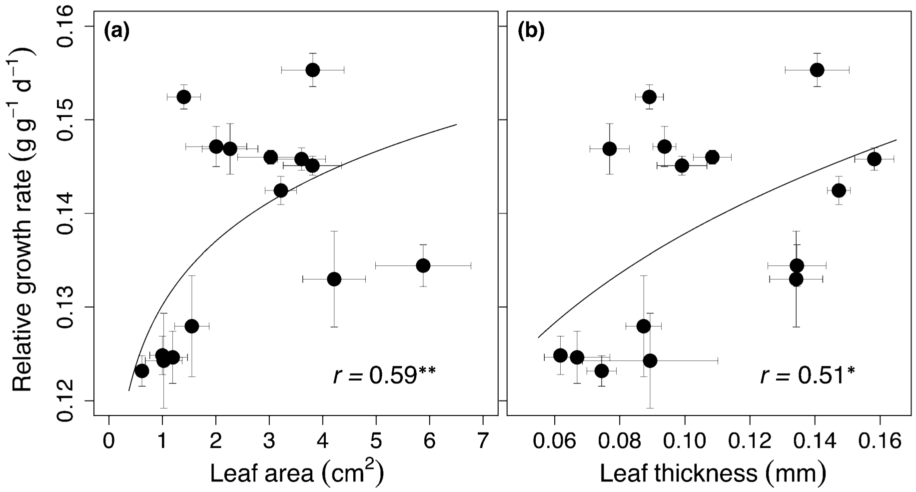

Fig. 4 Relationships of relative growth rate (RGR) with (a) leaf area and (b) leaf thickness for 15 Arabidopsis ecotypes grown in a glasshouse common garden. The *r*-values with significance are based on power law regressions accounting for kinship. *, $P < 0.05$; **, $P < 0.01$. Error bars indicate standard error.

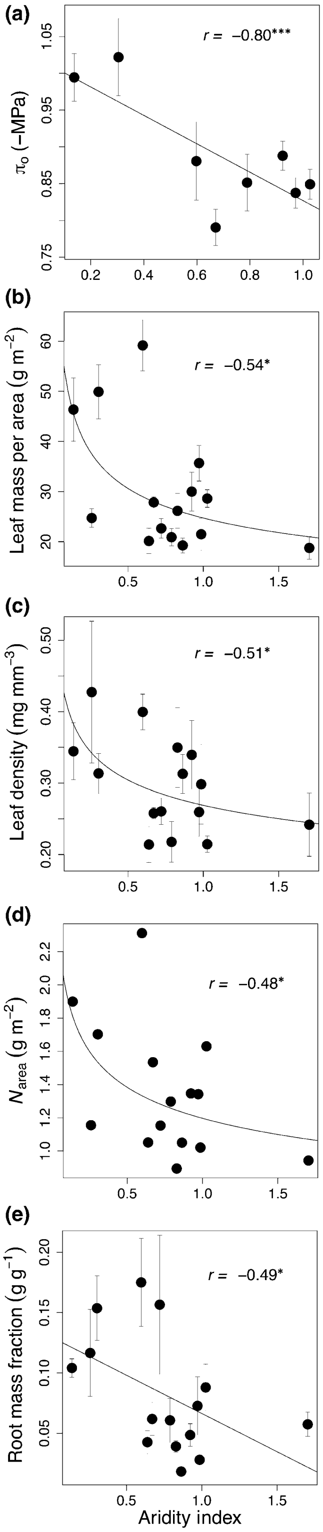

Fig. 5 Relationships of drought resistance traits with aridity index (AI; negatively related to climatic aridity) for Arabidopsis ecotypes grown in a glasshouse common garden, that is with (a) osmotic potential at full turgor (π_o; $n = 8$ genotypes), (b) leaf mass per area (LMA; $n = 15$ genotypes), (c) leaf density ($n = 15$ genotypes), (d) leaf nitrogen per area (N_{area}; $n = 15$ genotypes) and (e) root mass fraction (RMF; $n = 15$ genotypes). The *r*-values with significance are based on linear or power law regressions accounting for kinship. *, $P < 0.05$; ***, $P < 0.001$. Error bars indicate standard error.

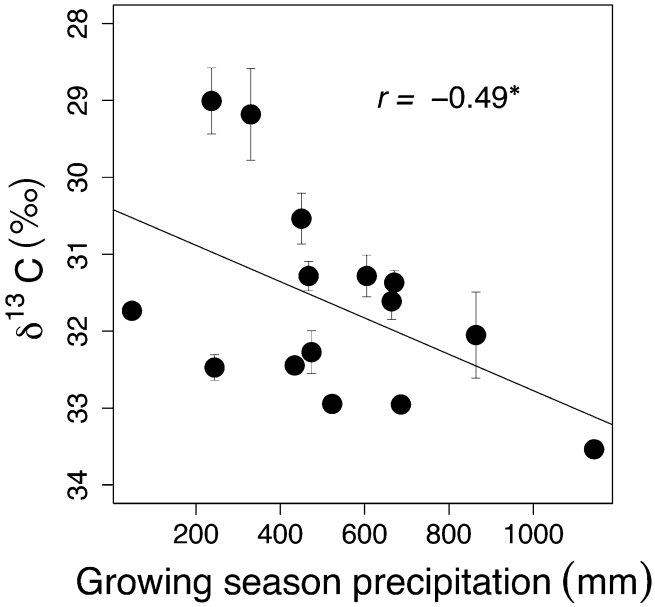

Fig. 6 Relationship of carbon isotope ratio (δ^{13}C) with growing season precipitation for 15 Arabidopsis ecotypes grown in a glasshouse common garden. The *r*-value with significance is based on a linear regression accounting for kinship. *, $P < 0.05$. Error bars indicate standard error.

independence of the traits that determine RGR or AGR from traits that contribute to adaptation to cold and dry climates. The lack of an intrinsic trade-off between RGR and climate variables is also consistent with Arabidopsis ecotypes adapting to cold or dry climates through stress resistance and/or stress avoidance, that is with some ecotypes partially mitigating a shorter growing period through rapid growth during a warm, moist growing period. The potential for ecotypes to adapt to withstand climate stresses without cost to growth capacity under high resource conditions would contribute to the large climate range of the species.

Decoupling of RGR from adaptation to cold and arid climates within Arabidopsis

While across the 15 ecotypes RGR was statistically independent of climate variables, there was a nonsignificant empirical trend for more rapid growth of ecotypes native to colder climates (Fig. 2a), which was consistent with that found for 60 ecotypes in the data of Atwell *et al.* (2010; Fig. S1c,d) and in our reanalysis of data from Vasseur *et al.* (2018) showing strong variation in RGR independent of MAT over most of the climate range, but relatively high RGR for ecotypes at the extreme coldest and hottest ends of the range (Fig. S1a). Our finding of the independence of RGR from MAP across ecotypes is consistent with both previous studies where RGR was determined as rosette area expansion (Atwell *et al.*, 2010; Vasseur *et al.*, 2018), and extends those findings to mass-based RGR. Overall, all data are consistent in showing that slow-growing, stress-tolerant ecotypes can be common in cold and relatively arid climates, and yet, also typical of these conditions are stress-avoiding ecotypes with moderate to high RGR that mitigate extreme temperatures and dry periods with rapid growth in the shorter favorable season.

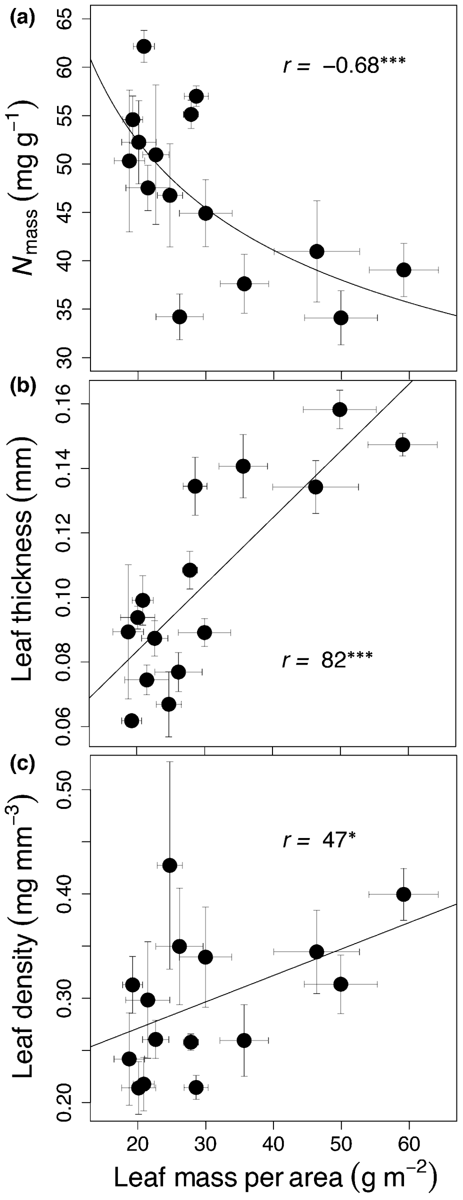

Fig. 7 Relationships of (a) leaf nitrogen per mass (N_{mass}) with leaf mass per area (LMA) and (b) leaf thickness (LT) and (c) leaf density (LD) with LMA, for which they are components (LMA = LT × LD) for 15 Arabidopsis ecotypes grown in a glasshouse common garden. The *r*-values with significance are based on linear or power law regressions accounting for kinship. *, $P < 0.05$; ***, $P < 0.001$. Error bars indicate standard error.

Notably, time to flowering (i.e. vegetative growth duration) is often considered a central trait in Arabidopsis ecology and evolution, linked with differences in growth and climate adaptation across ecotypes (Stinchcombe *et al.*, 2004; Lempe *et al.*, 2005; Mendez-Vigo *et al.*, 2011). We found a nonsignificant empirical trend for longer times to flowering in ecotypes adapted to cold climates, consistent with the weak association shown in studies using large numbers of ecotypes (Debieu *et al.*, 2013; Sartori *et al.*, 2019). We note that the specific relationship of RGR in a growth experiment with time to flowering would be dependent on the method of measurement. Studies that consider growth based on leaf area increment between germination and flowering (i.e. in which ecotypes grow for different time durations) typically report a *negative* correlation of AGR or RGR with time to flowering, as ecotypes that expand leaves more rapidly tend to do so over a shorter interval (Debieu *et al.*, 2013; Sartori *et al.*, 2019). In our study, we considered mass-based growth for a common time duration, including the full vegetative growth period to flowering and the production of fruits for all ecotypes, which is a typical approach used when comparing species for their growth (Kitajima, 1994; Sack, 2004). We found a significant *positive* relationship of RGR with time to flowering time, as expected given that later flowering corresponds to a longer period of vegetative growth. Overall, the balance of data suggests that growth is strongly linked with time to flowering, positively or negatively depending on the design of the growth measurements, and that flowering time is weakly associated with climate, and does not mediate a general association between RGR and adaptation to cold or aridity.

Decoupling of RGR from drought resistance and leaf economics traits

Several leaf traits were related to higher RGR and its most important component, LMF, including larger, thicker leaves with greater δ^{13}C, reflective of water-use efficiency, and higher Chl concentration. These relationships are consistent with ecotypes with larger plant sizes having more numerous and larger leaves, contributing to a higher LMF (Sack *et al.*, 2003). Furthermore, thicker leaves have a higher N_{area}, consistent with their greater number of cell layers, and would contribute to higher photosynthetic rates per leaf area (Ripullone *et al.*, 2003; Wright *et al.*, 2004). Yet, RGR was not correlated with the LES traits, SLA, N_{area} and N_{mass}. Across the ecotypes, high RGR was driven by multiple components, with high LMF being on average the most important causal driver. Thus, ecotypes may adapt to rapid RGR with a range of alternative trait combinations, that is via many-to-one mapping or trait multifunctionality (Marks & Lechowicz, 2006; Sack & Buckley, 2020).

Overall, drought resistance traits such as π_{o}, δ^{13}C, LMA and RMF were statistically independent of RGR, indicating no support for a trait-based mechanism for associations between RGR and stress tolerance.

Relationships of traits to cold and drought adaptation, and implications for seeking genes for stress tolerance

Several studies have used Arabidopsis as a platform to test the association of genetic variation with climate variables across populations (Li *et al.*, 2010; Hancock *et al.*, 2011; Lasky *et al.*, 2012; Bac-Molenaar *et al.*, 2016; Frachon *et al.*, 2018; Vasseur *et al.*, 2018; Ferrero-Serrano & Assmann, 2019), and sought to identify genes underlying water-use or drought tolerance (Ingram & Bartels, 1996; Mojica *et al.*, 2016; Exposito-Alonso *et al.*, 2018). Yet, thus far, many physiological traits most directly related to drought resistance have remained unquantified, such as π_{o}, which is a promising trait in explaining variation in drought resistance. Our results extend the theory of π_{o} diversification and confirm that π_{o} can

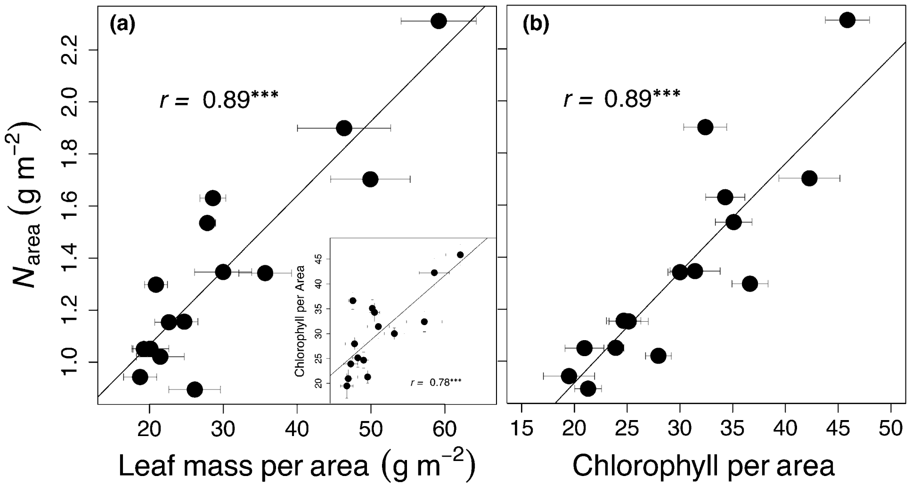

Fig. 8 Relationships of (a) leaf nitrogen per area (N_{area}) and Chl per area (inset panel) with leaf mass per area, and of (b) Chl per area with N_{area} for 15 Arabidopsis ecotypes grown in a glasshouse common garden. The *r*-values with significance are based on linear regressions accounting for kinship. ***, $P <$ 0.001. Error bars indicate standard error.

predict plant drought resistance across ecotypes. This builds upon previous work showing that turgor loss point (π_{TLP}), of which π_o is the main determinant, can predict drought resistance for diverse species across local habitat gradients or across biomes (Bartlett *et al.*, 2012b; Rosas *et al.*, 2019; Kunert *et al.*, 2021), across closely related species within a lineage (Fletcher *et al.*, 2018), and across cultivars or natural populations of given species (Mart *et al.*, 2016; Rosas *et al.*, 2019). While osmotic adjustment can influence π_{TLP} during drought, Bartlett *et al.* (2012b) found that across diverse species, osmotic adjustment capacity does not overwhelm the relationship of π_{TLP} with drought resistance, except in certain crops. Detailed quantification of π_o and osmotic adjustment has applications to crop breeding (Flavell, 2005; Liu, 2010; Chew & Halliday, 2011) and determining species' vulnerability to climate change (Exposito-Alonso *et al.*, 2018). The lack of an intrinsic trade-off between growth capacity and aridity adaptation implies substantial flexibility to enable crop breeding for combinations of rapid growth and climate tolerance.

This work is novel in showing that in addition to π_o, there were several other leaf and whole-plant traits including LMA, leaf density, RMF and δ^{13}C that increased with increasing aridity or precipitation across genotypes of Arabidopsis as predicted by published work across diverse species (Niinemets, 2001; Sack *et al.*, 2003; Wright *et al.*, 2004; Poorter *et al.*, 2009; Wang *et al.*, 2016). N_{area} showed the same trend, probably due to its tight positive correlation with LMA. N_{mass}, however, was negatively correlated with LMA, consistent with a fast-growing leaf, while the components of LMA, leaf thickness and density, and Chl/area all showed the expected positive trends with LMA (Niinemets, 2001; Poorter *et al.*, 2009).

Species-scale implications of the decoupling of RGR from adaptation to cold and aridity

The lack of a strong intrinsic, trait-based trade-off across ecotypes between RGR and adaptation to cold or arid climates has important implications for species-scale ecological processes. The decoupling of RGR and climate adaptation is consistent with both stress-tolerant and fast-growing avoidant strategies in ecotypes native to extreme climates. Based on ecological theory (A1), the decoupling of RGR from cold and drought adaptation would extend the distribution of Arabidopsis ecotypes with respect to temperature and water supply, and thus would contribute to a large species range, especially given the high gene flow that has been documented between Arabidopsis populations in Europe (excluding populations of the Iberian Peninsula; Alonso-Blanco *et al.*, 2016). Indeed, Arabidopsis occurs across much of Europe and Asia, and parts of North America (Alonso-Blanco *et al.*, 2016). Furthermore, ecological theory predicts that in the absence of a growth vs stress-tolerance trade-off, ecotypes could coexist across large climate gradients, and the range of the species overall would show greater resiliency in response to climate change (A1). The contribution of trait variation among ecotypes to species resilience to climate change is an important avenue for future research. Consistent with this hypothesis, a recent study of Arabidopsis leaves preserved in herbaria found that traits such as carbon isotope ratio and C : N ratio shifted with climate change (DeLeo *et al.*, 2020).

Drawing inferences despite limitations of the experimental design

Our study tested for associations between RGR and adaptation to native climatic cold and aridity across 15 diverse ecotypes measured in detail. Inference depends on several assumptions following previous literature, supported by the physiology of this model species. First, while the set of 15 diverse ecotypes would not represent the full range of growth and trait variation across the species, the power is sufficient to test for a general intrinsic association between growth and adaptation to climate cold and aridity (Sack, 2004; John *et al.*, 2013). Second, we considered the relationships of growth and traits measured in a common garden with cold and aridity of the native range. Growth and trait values measured in the common garden represent differences due to genetic variation between ecotypes achieved in high-resource conditions (Cordell *et al.*, 1998; Dunbar-Co *et al.*, 2009; Givnish & Montgomery, 2014; Mason & Donovan, 2015), minimizing plastic changes expected for plants in the field, which may adjust to abiotic and biotic conditions, including nutrient and water availability (Fernández & Reynolds, 2000). Third, we assumed that cold- and drought-tolerances are represented by the temperature and aridity of ecotypes' native ranges following Lubbe & Henry (2019) and Koehler *et al.* (2012), an assumption also supported by our analysis of the data of a published study on 211 Arabidopsis ecotypes, showing that survival in a drought experiment was correlated with climatic aridity in the native range (data of Exposito-Alonso *et al.*, 2018; Fig. S3). Fourth, traits were measured in this study in warm, well-watered conditions, and thus do not reflect potential acclimation, such as plants in drying soil allocating more strongly to deeper roots, higher LMA and lower osmotic potential (Huck *et al.*, 1986; Poorter *et al.*, 2009; Comas *et al.*, 2013; Bartlett *et al.*, 2014; Eziz *et al.*, 2017). However, previous studies show that despite the lack of plastic adjustments, the ranking of species or ecotypes in their resistance traits in well-watered conditions is generally representative of these rankings under droughted conditions (Sack, 2004; Ramirez-Valiente & Cavender-Bares, 2017; del Pozo *et al.*, 2020). In particular, for annual plants facing strong cold or drought, there would be limited potential for acclimation before experiencing stress that would slow growth (Bouzid *et al.*, 2019). Additionally, our finding that drought resistance traits measured in the common garden varied strongly and were associated with climatic aridity in the native range, yet were statistically independent of RGR and AGR, suggests that these stress resistance traits do not intrinsically constrain RGR or AGR. We note that this experimental design would be strongly complemented by future studies testing these assumptions for growth and stress resistance under field conditions.

Conclusions

Across ecotypes of Arabidopsis we found no support for a strict trade-off between RGR and cold or drought tolerance. We found that RGR was not overall limited by any single one of its components, or constrained by functional traits related to cold or drought resistance or avoidance. Based on ecological theory, the lack of a constraint on combinations of RGR and climatic stress resistance across ecotypes would contribute to the species' occupation of a large climatic range and resilience in response to climate change. The mechanistic independence of RGR from stress tolerance points to possible bases for improving crop stress tolerance without intrinsic cost to productivity.

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Author contributions

LS and CS designed the experiment, LRF and CS collected data, LRF, CF, MP, TNB and LS analyzed the data, LRF generated the figures and tables, LRF and LS wrote the manuscript, and all authors contributed to revisions.

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Data availability

The data used for this study are available in the supplementary materials published with this article.

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Appendix A1

The ecological consequences of within-species growth–stress tolerance relationships

We synthesized theory for the potential ecological consequences of growth-stress tolerance relationships (GSTRs) across ecotypes of a given species based on the published literature using a simple framework (Fig. A1). We consider a simple gradient of habitats from low to high climatic stress, where low climatic stress was assumed to provide favorable competitive conditions for most plants (Walter, 1979). Under a trade-off between maximum relative growth rate (RGR) and stress tolerance, ecotypes specializing in rapid RGR should dominate under favorable climates but would be replaced by stress-tolerant ecotypes under stressful climates (Fig. 1a). By contrast, under a positive coordination of RGR and stress tolerance, the same ecotypes should dominate across the climatic gradient (Fig. A1b). Finally, if RGR and stress tolerance are decoupled, ecotypes with any combination can exist, and thus ecotypes vary in their ranges across the gradient, with some confined to extremes and others to substantial ranges (Fig. A1c).

This framework provides a heuristic for prediction of how major ecological properties of the ecotypes and the species itself should vary under different GSTRs. We considered four properties: ecotype specialization in high vs low climatic stress niches, species differentiation into ecotypes across a climatic gradient, ecotype turnover across a climatic gradient, and the width of the species range across continuous habitat (Table A1).

Ecotype specialization in high vs low climatic stress niches is represented by the total numbers of ecotypes existing within individual habitats (i.e. considering each of the single three squares vertically in Fig. A1, or averaging across them). Thus, under a trade-off, there is high ecotype specialization, relative to under positive coordination or decoupling (Kneitel & Chase, 2004; Ostman *et al.*, 2014; Fig. A1; Table A1). Ecotype differentiation across a climatic gradient is represented by the total numbers of ecotypes existing across the entire gradient (i.e. summing ecotypes across the three squares vertically in Fig. A1), and would thus be moderate, low and high respectively under a trade-off, positive coordination and decoupling (MacArthur & Levins, 1967; Mouquet & Loreau, 2002; Kneitel & Chase, 2004; Farahpour *et al.*, 2018; Table A1). Ecotype turnover across a climatic gradient, assuming complete dispersal, is represented by the shift in species composition across the gradient (i.e. the change in species across the three squares vertically in Fig. A1) and would thus be high, moderate and low respectively under a trade-off, positive coordination and decoupling (Kneitel & Chase, 2004). An expectation for the width of the species range can be made, assuming continuous habitat and complete gene flow, given that gene flow would lead to a reduced range, because genetic adaptation to specific climates would be 'diluted' by genes across the range. Finally, the width of the range of the species is represented by the degree that specialist ecotypes occur at the extremes (i.e. differentiated ecotypes in the highest and lowest squares of the vertical columns in Fig. A1) and would be relatively small, large and moderate respectively under a trade-off, positive coordination and decoupling (Mayr, 1963; Antonovics, 1976; Kirkpatrick & Barton, 1997; Sexton *et al.*, 2011; Fig. A1; Table A1).

Climate change would be expected to influence these effects of GSTRs on the ecological properties of a species and its ecotypes. Assuming that climate change overall results in an increase of extreme habitat, one can hypothesize influences on each outcome

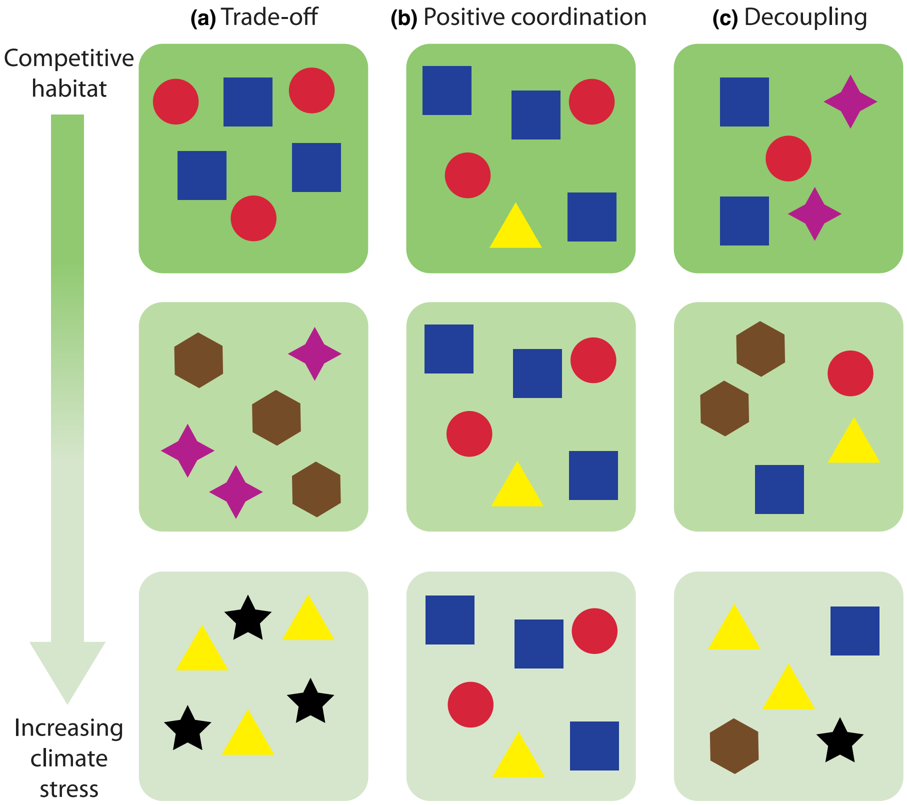

Fig. A1 Schematic diagram showing ecological implications of contrasting growth–stress tolerance relationships (GSTRs) for ecotypes of a species across a gradient from favorable climate (competitive habitat) to stressful climate. GSTRs include (a) trade-off, (b) positive coordination and (c) decoupling. Green squares represent locations of varying climatic stress and shapes represent different ecotypes.

Table A1 Synthesis of literature indicating theoretical influences of growth–stress tolerance relationships (GSTRs) on major ecological properties of a species and its ecotypes, including ecotype specialization in high vs low climatic stress niches, ecotype differentiation across a climatic gradient, ecotype turnover across a climatic gradient and the width of the species range across continuous habitat.

GSTR	(1) Ecotype specialization in high vs low climatic stress niches	(2) Ecotype differentiation across a climatic gradient	(3) Ecotype turnover across a large-scale resource gradient with continuous habitat given complete dispersal	(4) Species range across continuous habitat (assuming complete gene flow)
Trade-off	High–	Moderate–	High	Small–
Positive coordination	Low	Low	Low	Large–
Decoupling	Low–	High–	Moderate–	Moderate–
References	1, 2	1, 3, 4, 5	1	6, 7, 8, 9

Expectations are given for three GSTRs that have been hypothesized across ecotypes of given species, that is a trade-off between maximum relative growth rate (RGR) and stress tolerance, positive coordination of RGR with stress tolerance, and decoupling of RGR and stress tolerance. As climate change would tend to result in an increase in extreme habitat, expectations were also provided for the influence of climate change on each outcome; a minus sign indicates that climate change would decrease the hypothesized effect in a given category.
References: 1. Kneitel & Chase (2004); 2. Ostman *et al*. (2014); 3. MacArthur & Levins (1967); 4. Mouquet & Loreau (2002); 5. Farahpour *et al*. (2018); 6. Mayr (1963); 7. Antonovics (1976); 8. Kirkpatrick & Barton (1997); 9. Sexton *et al*. (2011).

(Table A1). In particular, greater representation of extreme climates would weaken some of the predicted trends associated with the influence of GSTRs across a climate gradient.

This theoretical synthesis is purely heuristic and untested, but illustrates the potentially strong impacts of GSTRs on the fundamental ecological properties of ecotypes and species, and their responses to climate change. This importance of GSTRs explains why they have been a focus of enormous research effort (summarized in Supporting Information Table S1) and the urgency of further research in testing for GSTRs, their mechanisms and ecological consequences.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Analyses of previously published data on the relationships between relative growth rate and climate across *Arabidopsis thaliana* ecotypes.

Fig. S2 Relationships of absolute growth rate and flowering times with climate across *Arabidopsis thaliana* ecotypes.

Fig. S3 Data supporting the correspondence of experimental drought tolerance with native climatic aridity across 211 *Arabidopsis thaliana* ecotypes.

Table S1 Table of previous studies investigating a growth–drought tolerance trade-off.

Table S2 AIC scores for fitted models of data from Vasseur *et al.* (2018).

Table S3 Individual and mean data for 15 focal genotypes of *Arabidopsis thaliana* varying in native moisture distribution grown in a common garden.

Table S4 Correlations with kinship for all traits and climate variables on raw and logged data for 15 focal genotypes of *Arabidopsis thaliana* varying in native moisture distribution grown in a common garden.

Table S5 Significant correlations with kinship for all traits and climate variables on raw and logged data for 15 focal genotypes of *Arabidopsis thaliana* varying in native moisture distribution grown in a common garden.

Table S6 Correlations with kinship for all traits and climate variables on raw and logged data for eight focal genotypes of *Arabidopsis thaliana* varying in native moisture distribution grown in a common garden.

Table S7 Significant correlations with kinship for all traits and climate variables on raw and logged data for eight focal genotypes of *Arabidopsis thaliana* varying in native moisture distribution grown in a common garden.