Research Paper

Temperature-related cline in the root mass fraction in East Asian wild radish along the Japanese archipelago

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Wild plants with a wide distribution, including those exposed to a wide variety of environmental conditions, may have variations in key functional traits relevant for agricultural applications. The East Asian wild radish (*Raphanus sativus* var. *raphanistroides*) is an appropriate model plant because it is widely distributed and has outstanding sink capacity as well as two cultivars within the species. Multiple common garden trials with 14 populations and three testing sites were conducted across the Japanese archipelago to quantify variations in yield and allocation. Significant inter-population variations and interaction effects with testing sites were detected for the root and shoot mass and the root mass fraction (RMF). While the rank order of the population changed drastically among sites and the variance components of genetic effects were small in yield traits (2.4%–4.7%), RMF displayed a large genetic variance (23.2%) and was consistently higher in the northern populations at all sites. Analyses revealed that the mean temperature of growing season of the seed origin was the most prominent factor explaining variation in RMF, irrespective of the sites. We concluded that the trait of resource allocation had a temperature-related cline and plants in cooler climates could invest more resources into their roots.

Key Words: wild species, inter-population variation, allocation, sink development, common garden trial, radish.

Introduction

Genetic diversity in crop plants is one of the fundamental factors toward further agricultural improvements (Dwivedi *et al.* 2016). In particular, standing genetic variation associated with key functional traits can be useful in adapting to future climate change. However, domestication has reduced the potential for such adaptation in cultivated species due to the loss of genetic diversity (Barrett and Schluter 2008). Wild species occupying a wide range of environmental niches are expected to possess substantial genetic diversity owing to adaptation to local environments (Barrett and Schluter 2008, Kawecki and Ebert 2004, Ridley and Ellstrand 2010). Therefore, evaluation of intra-specific variation in wild relatives provides relevant information that is directly and/or indirectly applicable to breeding strategies of cultivars that encourage diversification of the gene pool and expand the collection of germplasm

resources, as well as to determining the optimal traits in a given environment (Carputo *et al.* 2013, Katoh *et al.* 2015, Kaushik *et al.* 2016, Mazer and Wolfe 1992, Warwick *et al.* 2009).

Biomass yielding and resource allocation (sink-source balance) are fundamental functional traits in plant growth and are agriculturally important. Both traits are strongly controlled by environmental conditions, particularly the temperature and water availability of the growing habitat (Fitter and Hay 1981, Poorter *et al.* 2012). The genetic basis of differentiations in these traits among and within species is relatively well understood for herbaceous and woody plants (Lambers *et al.* 2008, Poorter *et al.* 2012). However, quantitating inter-population variation requires multiple common garden trials (Ishizuka and Goto 2012, Kawecki and Ebert 2004, Ridley and Ellstrand 2010). In such trials, the adaptive potential of detected variations can be evaluated by demonstrating the clinal trends across specific geographic or environmental factors related to the origin of the tested populations. In this context, studies have found that plant reproductive traits, such as the timing of flowering exhibit clinal trends that reveal local adaptation (Han *et al.* 2016, Kang *et al.* 2016, Olsson and Ågren 2002,

Stinchcombe *et al.* 2004, Weber and Schmid 1998, Yoshie 2007). For many species, northern populations or populations from cooler climates tend to reproduce or cease growth earlier, given the trade-off between the two. Similar genetic clines have also been reported for growth potential. Populations from cooler climates have exhibited smaller biomass yields or plant sizes in herbaceous plants including: *Arabidopsis* (Li *et al.* 1998), goldenrods (Weber and Schmid 1998), soft snow-grass (Byars *et al.* 2007), mountain garland (Jonas and Geber 1998), and certain woody plants, such as Scots pine (Rehfeldt *et al.* 2002), Norway spruce (Skrøppa and Magnussen 1993), and Sakhalin fir (Ishizuka and Goto 2012). There are admixture reports concerning resource allocation; interspecific variation in herbaceous species has been demonstrated along precipitation (Schenk and Jackson 2002) or altitudinal (Körner and Renhardt 1987) gradients in their habitat, whereas inter-population variation has been reported along the latitude of origin for Scots pine (Oleksyn *et al.* 1992) and the altitude of origin for Norway spruce (Oleksyn *et al.* 1998). In general, relevant variations in biomass yielding and resource allocation are expected in plant species with wild relatives.

Radish (*Raphanus sativus* L., $2n = 18$) seems an appropriate model plant since this species has two major cultivated varieties, *R. sativus* L. var. *sativus* and *R. sativus* L. var. *hortensis* Becker (Asian cultivated radish; daikon) and one wild variety, *R. sativus* L. var. *raphanistroides* Makino (East Asian wild radish). Furthermore, the outstanding root development of radish is an agronomically important trait, and sink-source balance differs among local cultivars (Fujieda 1993, Iwata *et al.* 1998, Sugiura *et al.* 2015). The extreme example is the 'Sakurajima' cultivar, which develops a huge root yielding 10–20 kg of biomass (Fujii 1977). Recently, the regulation of root development has been reported by using an advanced molecular approach (Kim *et al.* 2016, Mitsui *et al.* 2015), and a potential future study could be to investigate the genetic variation associated with root yielding or allocation.

The East Asian wild radish likely harbors abundant and useful intra-specific variations owing to its wide distribution from southern sub-tropical to northern sub-boreal Asia (Kaneko *et al.* 2011). Within its main habitat, sand beaches, this wild radish displays substantial root elongation (Kim *et al.* 2016). Already, inter-population genetic variations and diversity across its distributed range have been well recognized in the traits of timing of flowering (Han *et al.* 2016) and leaf morphology (Yamaguchi 1987), as well as shown in molecular studies (Han *et al.* 2015, 2016, Huh and Ohnishi 2001, Lü *et al.* 2008, Ohsako *et al.* 2010, Wang *et al.* 2008, Yamane *et al.* 2009). From these molecular studies, consistent south-to-north divergences, sometimes with a latitudinal cline (Yamaguchi 1987), appear to be conserved in this species. However, the magnitude and extent of inter-population variations in root yielding and allocation are poorly understood.

In the present study, multiple common garden trials using native populations of East Asian wild radishes were conducted, and inter-population variation in root developmental traits was explored. Our aim was to reveal (1) the magnitude of differences in biomass yield and allocation among populations and (2) the candidate geographic and environmental factors responsible for the observed variation and their clinal trends.

Materials and Methods

Plant materials

The East Asian wild radish is found in coastal sandy areas and has a winter annual life cycle, sometimes forming high-density patches (Kaneko *et al.* 2011). Along the Japanese archipelago, we selected thirteen populations from both the Pacific Ocean and the Japan Sea coastal areas (**Fig. 1**). All populations were located on sandy beaches far from urban areas to reduce the chance that the origin of the selected population was recently escaped cultivars (Aoba 1988, Fujieda 1993).

Additionally, one inland population was selected from Lake Biwa, which is a well-known ancient freshwater lake that many coastal plants live in (Kitamura 1968). In total, we selected 14 populations for seed origins and numbered them from south to north as RsR01–14 (**Fig. 1**, **Table 1**). Among the most distant populations (RsR01 and RsR14), the latitudinal and longitudinal linear distances were approximately 1,500 km and 1,100 km, respectively. Mature
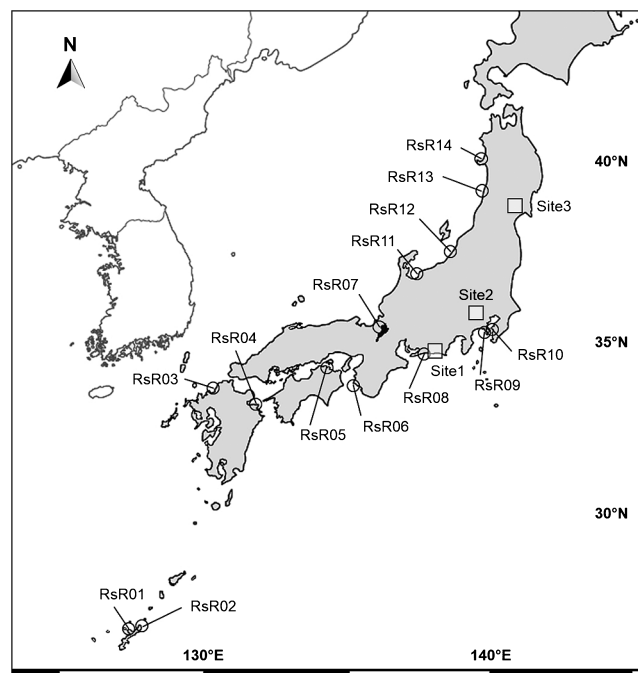

Fig. 1. Map of Japan showing the locations of seed source populations of East Asian wild radish and the sites of the multiple common garden trials. The 14 seed populations are labelled RsR01–14 along the Japanese archipelago.

Table 1. List of the populations of East Asian wild radish (*Raphanus sativus* var. *raphanistroides*) in Japan, with corresponding geographic information and environmental variables

| Code | Collection site | | Latitude (°N) | Longitude (°E) | Annual temperature (°C) | Growing season (Dec.–Apr.) | | |
	prefecture	location				Temperature (°C)	Precipitation (mm)	Days <5°C (days)
RsR01	Okinawa	Nagahama	26.706	127.945	22.6	18.0	659	0
RsR02	Okinawa	Hedo	26.864	128.264	20.7	16.1	871	0
RsR03	Fukuoka	Saitozaki	33.649	130.350	17.0	9.7	428	0
RsR04	Oita	Isozaki	33.244	131.785	16.4	9.2	386	12
RsR05	Kagawa	Aji	34.379	134.163	16.3	8.5	282	34
RsR06	Wakayama	Ao	33.906	135.068	16.5	9.1	352	21
RsR07	Shiga	Imatsu	35.434	136.041	13.7	5.6	719	94
RsR08	Shizuoka	Kosei	34.676	137.499	16.3	9.1	505	19
RsR09	Kanagawa	Hayama	35.253	139.583	15.8	9.1	450	0
RsR10	Chiba	Futtsu	35.313	139.796	15.9	9.1	556	3
RsR11	Toyama	Kurobe	36.867	137.416	13.6	5.7	1024	94
RsR12	Niigata	Teratomari	37.696	138.786	13.4	5.5	768	96
RsR13	Akita	Nikaho	39.188	139.906	12.7	4.9	584	106
RsR14	Akita	Oga	40.029	139.914	11.0	2.7	520	123

Temperature, a simple mean of the daily averages; Days <5°C, the number of days with a daily minimum temperature of <5°C.
Listed environmental variables were calculated from data averaged for 30 years (1981–2010) obtained from the Japan Meteorological Agency.

seeds were collected throughout each of the populations from more than a dozen adults in summer 2012 and stored in a dark and moisture-free chamber until use. Currently, all remaining seeds have been deposited in College of Bioresource Sciences, Nihon University, and are available for research use.

A variety of cultivated radish 'Tokinashi' (Tohoku Seed Co. Ltd., Japan) was used as the control.

Common garden trials

Three experimental fields were established from south to north (site 1, site 2 and site 3; **Fig. 1**). Multiple common garden trials were conducted at these sites. Site 1 was a coastal sandy field belonging to the Ecohydrology Research Institute, the University of Tokyo Forests, Shizuoka Prefecture (34.6821°N, 137.5729°E). This site was an open field surrounded by coastal pine forests. Site 2 was composed of nursery soil and located at the University of Tokyo Tanashi Forest, Tokyo Prefecture (35.7370°N, 139.5371°E). Site 3 was a crop experimental field for the crop of the Institute of Genetic Ecology, at Tohoku University, Miyagi Prefecture (38.4617°N, 141.0935°E).

At each site, three replicates were set in three furrows. At each replicate, bulk seeds from each population were sown in an incomplete randomized block design. Three rows were assigned for one population, and all rows were arrayed randomly. Irrigation and weeding were performed when necessary. After approximately 70 days of growth, plants were harvested. The dates of seed sowing to plant harvesting and total days of growth were 19 September to 3 December 2012 (75 days) at site 1, 2 October to 12 December (71 days) at site 2 and 13 September to 22 November (70 days) at site 3. All plants were harvested at site 1

because of high mortality, primarily due to larval attack, whereas 10 and 5 plants were harvested per population in each replicate at sites 2 and 3, respectively.

According to the digital loggers (HOBO; Onset Computer Corporation, Bourne, MA, USA), mean air temperature, ground temperature and effective day length (hours with >40 μmol m⁻² s⁻¹) during the entire growth period were 18.4°C, 20.7°C, and 9.7 h at site 1; 14.3°C, 17.3°C, and 8.8 h at site 2 and 16.6°C, 17.3°C, and 10.3 h at site 3, respectively. Soil N contents (NH₄ and NO₃) of sites 1, 2 and 3 were 12.9 ± 6.4, 16.7 ± 5.3 and 16.3 ± 6.7 mg/kgDW, respectively, according to the method by Doane and Horwáth (2003).

Harvested plants were separated into aboveground parts (hereafter, shoots) and the root. After drying for more than 3 days in an oven, the samples were weighed. The root mass fraction (RMF) was calculated as the root mass divided by the total biomass.

At the time of harvesting, the maturity stages of each plant were also recorded. In three sites, no flowering plants were observed. Bolting plants were recorded only in site 3, and others were all rosette plants without bolting. The number of bolting plants was limited (10% of all those in site 3), and the observed populations were limited to RsR01 and RsR02. We therefore did not conduct a statistical analysis of the relationship between maturity stage and biomass yielding or resource allocation.

Data analysis

We focused on three traits for analyses: the root mass, shoot mass and RMF and we quantified the effects of seed origin and growing site as well as the interaction effect between them on the three traits. All statistical analyses

were performed using R 3.6.1 (R Core Team 2019). The control cultivar 'Tokinashi' was excluded from analyses owing to the lack of the parameters associated with the seed origin and its large morphological differences with the wild radish.

For each trait, analysis of variance (ANOVA) was conducted using the model, as follows:

$$Y_{ijk} = \mu + \text{origin}_i + \text{site}_j + \text{replication}_{jk}(\text{site}_j)$$
$$+ \text{origin}_i \times \text{site}_j + \varepsilon_{ijk}, \quad (1)$$

where Y_{ijk} is the values of the target trait of the ith seed origin (i; 1–14) in the kth replication (k; 1–3) set within the jth testing site (j; 1–3), μ is the general mean, origin$_i$ is the effect of the ith seed origin, site$_j$ is the effect of the jth testing site, replication$_{jk}$ is the nested effect of the kth replication set within the jth site and ε_{ijk} is the residual error. This model is treated as nested-ANOVA. The variance components for each trait are additionally estimated using VarCorr function in lme4 package.

For each trait, the factors relating to the effects of seed origin were determined using generalized linear mixed models, as follows:

$$Y_{ijk} = f(z_{ijk}) + \varepsilon(\text{replication}_{jk}) + \varepsilon_{ijk}, \quad (2)$$

where $f(z_{ijk})$ is the fixed effect for estimating the trait Y_{ijk}, $\varepsilon(\text{replication}_{jk})$ is the random effect of the kth replication in the jth testing site and the model has a normally distributed error structure with an identity link function of z. We first assumed a simple model in which the component of z only represented the effect of testing sites, that is, $z_{ijk} = \text{site}_j$. This is denoted as the "null model". Next, we incorporated the effects of the geographic and environmental factors of the seed origin into the model, as the explanatory variable x. The linear response to x was assumed as well as the interactive effect of x with the growing conditions. In addition, we assumed the non-linear response of the target traits to x. Y_{ijk} would possess the characteristic which increase exponentially with increasing/decreasing x (Rehfeldt et al. 2002), or have the optimal value in the range of x that is well recognized in the temperature response of photosynthesis in C3 plants (Hikosaka et al. 2006). These inclusive responses can be incorporated into the model by the quadratic equation and subsequent selection procedures of the model components. We then set the model containing whole candidate components into z. This model is denoted as the "full model", as follows:

$$z_{ijk} = x_i + x_i^2 + x_i \times \text{site}_j + x_i^2 \times \text{site}_j + \text{site}_j, \quad (3)$$

where x_i is the variable relating to the geographical and/or environmental conditions of the ith seed origin. The full model was formulated by a quadratic equation of x_i with the interaction between x_i and site$_j$.

For the value x, we set five candidate factors. There were two geographic and three environmental variables (**Table 1**): 1) latitude and 2) longitude where the seeds were collected; 3) mean temperature of seed origin among the growing season (December–April); 4) sum of precipitation among the growing season; and 5) number of days with a daily minimum temperature of <5°C during the growing season (hereafter, days <5°C), quantitating the unfavorable conditions below the base temperature for radishes (Reeves et al. 1981). For the latter three variables, we used data averaged for 30 years (1981–2010) at the nearest meteorological station to the seed collection sites (data were obtained from the Japan Meteorological Agency; http://www.jma.go.jp/). Each variable was calculated by a simple mean or sum of the daily values from December 1 to April 30. We treated these five variables as the independent models with respect to the multicollinearity because of strong correlations among each of the variables (data not shown).

The selection procedures of the model components were required because, for some factor, there was a possibility that the full model was not suitable due to the liner relationship or lack of association with the target trait. It was also required to determine the most relevant factor among all candidate x. Thus, model analyses were subsequently conducted using two-step selection procedures. For the first step to determine the best components of z against each x, backward selections of variables from the full models were conducted using the Akaike information criterion (AIC) values (Johnson and Omland 2004). The fittings of selected models were checked comparing those of the null models using likelihood-ratio tests. For the second step to determine the best-fit model among all candidate x, the model with the lowest AIC value was selected. We then quantified the clinal trends in the selected variables. These analyses were performed using lme4, MuMIn and effects packages.

Results

Inter-population variations

The population means of the root and shoot mass are presented for each site in **Fig. 2a–2c**. At site 1, a 4.5-fold difference was observed in the total biomass between the population yielding the largest plants (RsR12) and that yielding the smallest plants (RsR03) (**Fig. 2a**). Note that RsR14 at site 1 was omitted from the following analysis because only one individual survived. Similarly, there was a 2.9-fold difference (RsR08 vs. RsR02) and a 5.9-fold difference (RsR07 vs. RsR06) at sites 2 and 3, respectively, in the biomass between populations with the largest and smallest plants (**Fig. 2b**, **2c**).

Both the root and shoot mass were remarkably small at site 1 compared with those at sites 2 and 3 (**Fig. 3a**, **3b**). The overall means of the root and shoot mass at site 1 were 0.32 ± 0.40 g and 0.91 ± 1.09 g, respectively. The means of the root and shoot mass at site 2 were 3.47 ± 5.54 g and 13.60 ± 19.59 g, respectively. Similarly, these means at site 3 were 4.62 ± 4.37 g and 13.90 ± 11.34 g, respectively.
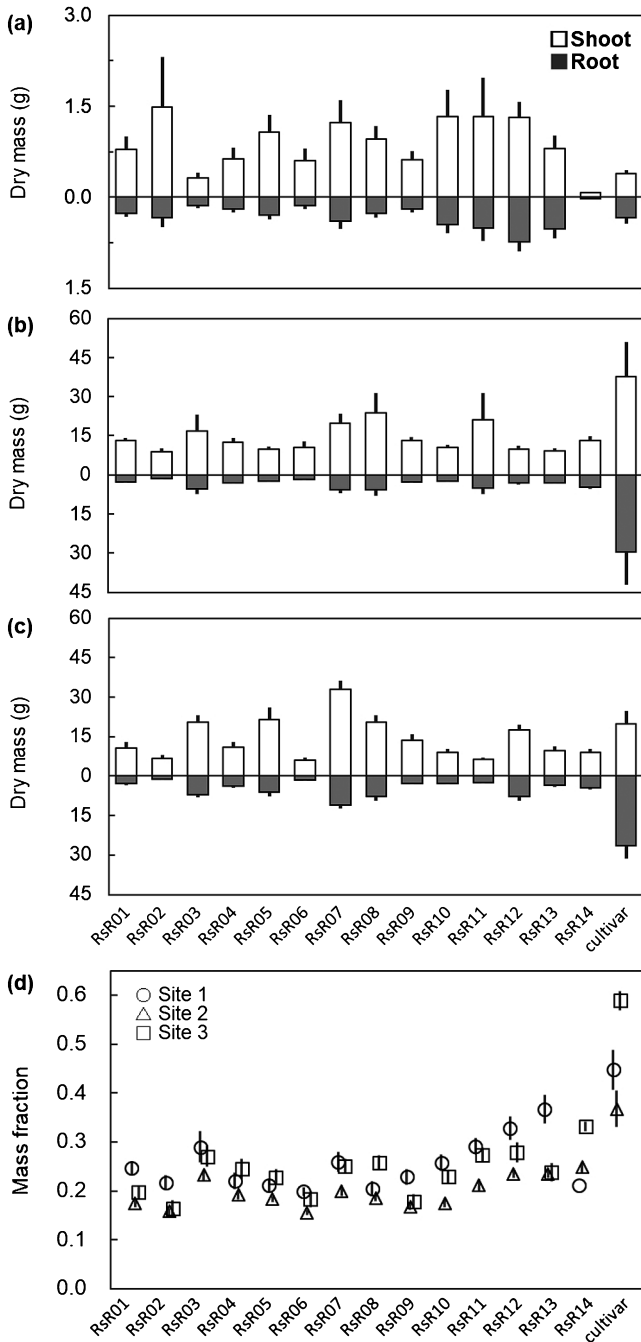
Fig. 2. The root and shoot (aboveground parts) dry mass of East Asian wild radish, grown at site 1 (a), site 2 (b), site 3 (c) and the root mass fraction (RMF) at all sites (d). Population averages with standard error (SE) are shown by symbols, and symbol legends are shown in panels a and d. Cultivated radish (*R. sativus* var. *hortensis* 'tokinashi') was used as the control.

Among the three sites, there were drastic shifts in the rank order of the population means of the root and shoot mass (**Fig. 3**).

In contrast with the yields described above, the population means of RMF (root mass fraction) were similar among the three sites (**Figs. 2d**, **3c**). The overall means of

RMF at site 1, 2 and 3 were 0.247 ± 0.079, 0.197 ± 0.052 and 0.238 ± 0.077, respectively. The maximum differences in RMF were 0.168 (RsR13 vs. RsR06) at site 1, 0.092 (RsR14 vs. RsR06) at site 2 and 0.166 (RsR14 vs. RsR02) at site 3. Because the rank order of the populations was nearly stable among the sites, northern populations, such as RsR11, RsR12, RsR13 and RsR14 were high-ranking populations at all sites (**Fig. 3c**).

These growth characteristics for wild radish were clearly different from those of cultivated radish, especially for RMF (**Fig. 2**). Biomass allocation to the root was substantial; RMF of the cultivar ranged from 0.368 (site 2) to 0.589 (site 3). Much greater yield was detected in the cultivar at sites 2 and 3 (**Fig. 2c**, **2d**), while the yield was moderate at site 1 (**Fig. 2a**).

ANOVA revealed the significant effects of the seed origins, testing sites and their interaction for all three target traits (**Table 2**). The degrees of the variances of effects were similar between the root and shoot traits; variances of the origin (4.7% and 2.4%) and the interaction (5.3% and 3.3%) were smaller than those of the site (17.4% and 18.3%). However, for RMF, the variance of the origin was 23.2%, which was higher than that of the site (13.7%) and the interaction (5.8%). The studied species possessed relatively great inter-population variation in RMF.

Relating factors for clinal trends

Model selection analyses detected the best models for root mass and RMF with significantly superior fittings compared with those of the null models (**Table 3**). On the contrary, for shoot mass, none of the candidate variables were effective for model fitting, and the best model was equal to the null model. Therefore, the model only estimated site-dependent differences (**Table 3**; see also **Fig. 4d–4f**). Estimated parameters for those best models are presented in **Supplemental Table 1**.

According to the results for root mass, the component of the best-fit model was precipitation of seed origin and testing site and their interaction (**Table 3**). Due to the significant interaction effect, predicted trends of the model along with precipitation were not consistent among sites; slightly better root yield was estimated for populations derived from regions with more precipitation at site 1, and conversely, root yielding was slightly higher for populations with less precipitation at sites 2 and 3 (**Fig. 4c**).

According to the results for RMF, the component of the best-fit model was temperature; site was excluded from the model (**Table 3**). The AIC value of the subsequent model that had a variable of (days <5°C)^2 was slightly higher than that of the best model. The best model predicted that populations derived from cooler climates would exhibit higher RMF values (**Fig. 4h**).

Although geographic variables were not selected as the best models for root mass and RMF, latitude rather than longitude was a more useful parameter in predicting both traits (**Table 3**). At all sites, populations derived from
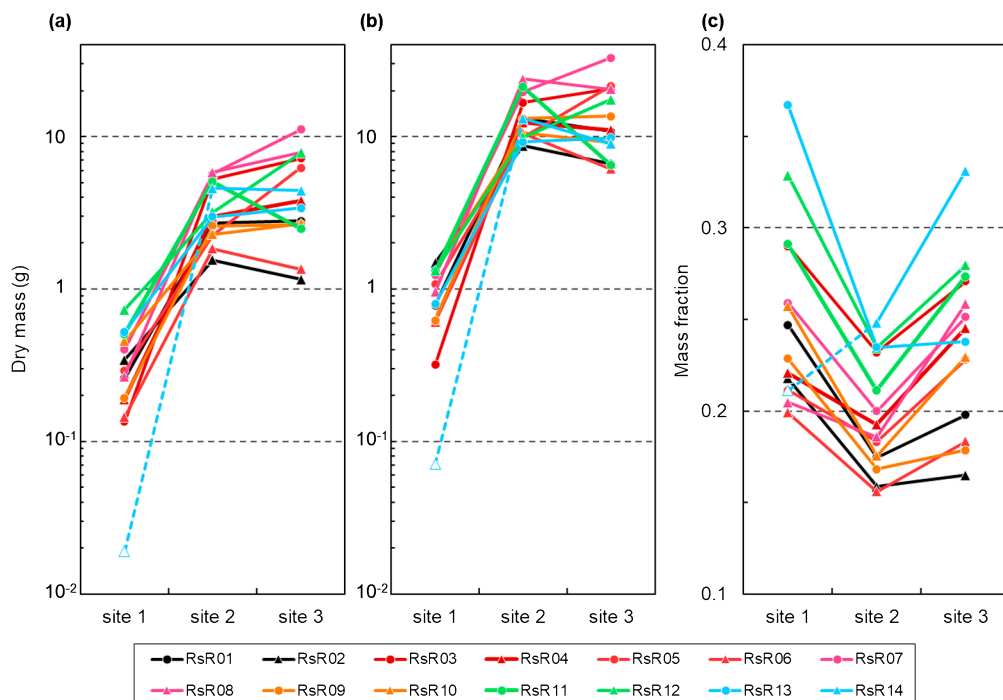
Fig. 3. Changes in the population means of three traits among the testing sites: (a) the root dry mass; (b) the shoot dry mass; and (c) the root mass fraction (RMF). The values for population RsR14 at site 1 are shown by open symbols with dashed lines because there was only one survivor.

Table 2. Results of nested analysis of variance of the root and shoot dry mass and the root mass fraction (RMF) of wild radish in multiple common garden experiments

Effect	DF	Root			Shoot			RMF		
		MS	F-value	Variance	MS	F-value	Variance	MS	F-value	Variance
Origin	13	106	5.7 ***	4.7	735	3.4 ***	2.4	0.068	23.2 ***	23.2
Site	2	823	18.0 ***	17.4	9436	21.9 ***	18.3	0.178	11.9 ***	13.7
Replication (site)	6	41	2.2 *	1.1	402	1.9	0.9	0.019	6.5 ***	4.5
Origin × site	24	40	2.2 ***	5.3	363	1.7 *	3.3	0.008	2.7 ***	5.8
Error	656	18		71.4	214		75.1	0.003		52.7

Fixed effects in all models were seed origin (origin), growth testing sites (site) and their interaction. Effect of the replication incorporated a priori in each of the sites was set as a nested effect. Variance components of effects are represented as well as degrees of freedom (DF), mean square (MS) and F-value (F). Statistical probabilities are as follows: *, $p < 0.05$; ***, $p < 0.001$.

Table 3. Selected model components and AIC values for each candidate factor (x) and for each trait

x	Root		Shoot		RMF	
	selected z	AIC	selected z	AIC	selected z	AIC
(null)	site	1948.7	site	1790.6	site	−1812.0
Latitude	x + site	1920.4 *	site	1790.6	x	−1904.5 -
Longitude	x + site	1935.5 *	site	1790.6	x	−1835.5 -
Temperature	x + site	1918.8 *	site	1790.6	$x + x^2$	**−1942.5** *
Precipitation	$x + x^2 + x \times$ site + site	**1913.6** *	site	1790.6	site	−1812.0
Days <5°C	x + site	1916.5 *	site	1790.6	x^2	−1941.7 -

Bold values with underline represent the lowest values among the traits, which is defined as the best-fit model after the model selection.
RMF, root mass fraction; *, statistically significant ($p < 0.05$) by the likelihood-ratio test compared with the null model; -, not tested due to the same number of variables (lacking the degree of freedom).
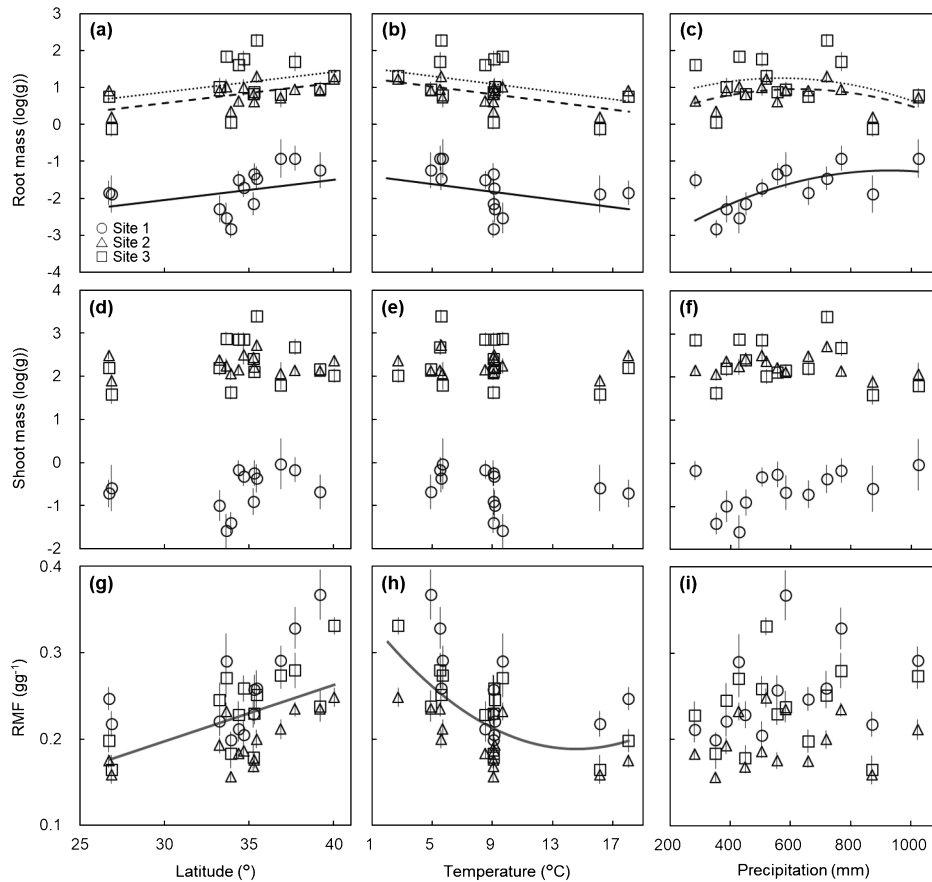
Fig. 4. The relationship between three traits obtained from common garden trials (the root and shoot dry mass and the root mass fraction) and three variations of seed origin (latitude, average temperature in the growing season and total precipitation in the growing season). Population averages with standard error are shown by symbols, and symbol legends are shown in panel a. The results of model fittings are also represented. For the trait of root mass (a, b, c), selected models show the difference of sites as solid, dashed and dotted lines, which refer to the regression lines for sites 1, 2 and 3, respectively.

higher latitudes tended to exhibit higher RMF values with better root yielding (**Fig. 4a**, **4g**).

Discussion

Multiple common garden trials can help evaluate inter-population variation and the ecological and agronomical significance of functional traits. In the present study, we focused on the traits of root and shoot yield and biomass allocation to the root of East Asian wild radish. All the three traits showed substantial inter-population variations and significant interactive effects with the growing conditions, that is, "origin × site" (**Table 2**). RMF, particularly, displayed a large genetic variance (23.2% of the total variance) compared with the root and shoot mass (4.7% and 2.4%, respectively). Furthermore, the effect of origin on RMF was larger than that of the environment or the interaction (**Table 2**); the rank order of the population was nearly stable among sites (**Fig. 3c**). These results indicate a strong genetic effect on inter-population variation in the mass fraction.

Based on the model analysis, the mean temperature of

the growing season of the seed origins was the strongest variable, whereas the AIC difference of the second model that had a variable of (days <5°C)^2 was small (**Table 3**). These results indicate that temperature-related conditions of origin are responsible for the genetic divergence of RMF. Furthermore, a consistent clinal variation was detected regardless of the experimental site. Populations from cooler regions tended to have a higher RMF along the Japanese archipelago (**Fig. 4h**). Note that the cline represented by the best model was not a linear but a quadratic trend. Similarly, in previous studies involving common garden trials, higher resource allocation to roots was found in cooler regions of Scots pine (Oleksyn *et al.* 1992) and Norway spruce (Oleksyn *et al.* 1998). Channeling resources to roots is a recognized adaptive response to stressful conditions (Fitter and Hay 1981). Because cooler regions tend to have longer periods when conditions are unfavorable during growth episodes and pose the risk of frost exposure, plants growing in such regions would benefit from investing resources in sink organs through root development. This may be a reasonable explanation for the temperature-related cline detected in our studied species.

The consistency of variations in the other traits of wild radish is also worth highlighting. Although model fitting was inferior to the temperature-related factors, a significant relationship between RMF and latitude of origin was evident in the present study; a higher RMF was observed in the northern populations (**Fig. 4g**). Latitudinal cline or southern-to-northern population divergence has been found in leaf morphology (Yamaguchi 1987), leaf and stem characteristics (Han *et al.* 2015), and the timing of flowering (Han *et al.* 2016). As with the radish, temperature is a critical factor for the initiation of flowering (Nie *et al.* 2016, Yoshida *et al.* 2010). This phenological response has an adaptive role for herbaceous species relative to local temperature, particularly against unfavorable low temperatures (Andrés and Coupland 2012). We therefore hypothesized that the wild radish along the Japanese archipelago would exhibit local adaptation in the mass fraction and phenological response. Further research is needed to validate the association of temperature in its native habitat with radish traits.

A consistent geographic or environmental cline was not recognized for the yield traits. Although root and shoot mass exhibited significant inter-population variations, genetic divergence of shoot yielding could not be explained by candidate factors. Genetic divergence of root yield was associated with precipitation of origin based on the best model, but the clinal trend was evident only in the testing site 1 (**Fig. 4c**). Due to the interaction between seed origin and testing site, the association of the drought condition of origin was complicated. Based on our results, inter-population variation in the yield traits appeared to be independent of adaptive genetic diversity across the environmental gradient. In general, growing conditions strongly affected plant growth rather than the difference of genotypes; for example, plants experiencing weak and/or moderate drought conditions had higher yielding potential with a faster growth rate (Fitter and Hay 1981, Poorter *et al.* 2012).

Environmental effects can also be evaluated; the considerable variance in components of these effects (17.4%, 21.9% and 13.7% for root mass, shoot mass and RMF, respectively) mean that the measured traits were influenced by environmental conditions of growing sites. Biomass yielding at site 1 was poor, whereas growth season, light and nutrient conditions appear to be similar among the three experimental sites. This may be explained by higher abiotic/biotic stresses at site 1, namely sandy soil and insect attack during the growth experiment. Nevertheless, the growth suppression of the wild radish was relatively less than that of the cultivated radish (**Fig. 2**). These findings indicate a higher resistance capacity of the wild radish to such stressful conditions and their potential as a genetic resource to relative cultivars. Already, agricultural application of resistance for abiotic/biotic stresses in wild relative species to cultivars has been reported in potato, *Solanum* (Carputo *et al.* 2013).

Thus, our study suggests that the East Asian wild radish exhibits large inter-population variation, in particular, a temperature-related cline in biomass allocation. This clinal trend indicates a genetic response to the local habitat. This information could be beneficial for constructing a future breeding strategy for radishes, e.g., when evaluating and using local cultivars throughout Japan or collecting and introducing relevant genetic resources. Further research is needed to understand the genetic basis of the inter-population variation in the wild radish. Genome screenings, as conducted in other radish species (Kim *et al.* 2016), could be the way forward for identifying genes responsible for root development in *Raphanus*.

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