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

Wataru Ishizuka*1), Kouki Hikosaka2), Motomi Ito3) and Shin-Ichi Morinaga4)

1) Forestry Research Institute, Hokkaido Research Organization, Kosyunai, Bibai, Hokkaido 079-0198, Japan
2) Graduate School of Life Sciences, Tohoku University, Aoba, Sendai, Miyagi 980-8578, Japan
3) Graduate School of Arts and Sciences, the University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan
4) College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan

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,
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

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
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 | Location       | Temperature (°C) | Precipitation (mm) | Days <5°C (days) |
|--------|-----------------|----------------|------------------|--------------------|------------------|
| RsR01  | Okinawa         | Nagahama       | 20.7             | 871                | 0                |
| RsR02  | Okinawa         | Hedo           | 17.0             | 428                | 0                |
| RsR03  | Fukuoka         | Saitozaki      | 16.4             | 386                | 12               |
| RsR04  | Oita            | Isozaki        | 16.3             | 282                | 34               |
| RsR05  | Kagawa          | Aji            | 16.5             | 352                | 21               |
| RsR06  | Wakayama        | Ao             | 13.7             | 719                | 94               |
| RsR07  | Shiga           | Imatsu         | 16.3             | 505                | 19               |
| RsR08  | Shizuoka        | Kosei          | 15.8             | 505                | 19               |
| RsR09  | Kanagawa        | Hayama         | 16.3             | 15.5               | 12               |
| RsR10  | Chiba           | Futtsu         | 15.9             | 556                | 3                |
| RsR11  | Toyama          | Kurobe         | 13.6             | 1024               | 94               |
| RsR12  | Niigata         | Teratomari     | 18.3             | 768                | 96               |
| RsR13  | Akita           | Nikaho         | 12.7             | 584                | 106              |
| RsR14  | Akita           | Oga            | 11.0             | 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/kg DW, 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:

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

where $Y_{ijk}$ is the values of the target trait of the $i$th seed origin ($i$; 1–14) in the $k$th replication ($k$; 1–3) set within the $j$th testing site ($j$; 1–3), $\mu$ is the general mean, origin$_i$ is the effect of the $i$th seed origin, site$_j$ is the effect of the $j$th testing site, replication$_{jk}$ is the nested effect of the $k$th replication set within the $j$th site and $\varepsilon_{ijk}$ 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:

$$Y_{ijk} = f(z_{ijk}) + \varepsilon(\text{replication}_{jk}) + \varepsilon_{ijk},$$  \hspace{1cm} (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 $k$th replication in the $j$th 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:

$$z_{ijk} = x_i + x_i^2 + x_i \times \text{site}_j + x_i^2 \times \text{site}_j + \text{site}_j,$$  \hspace{1cm} (3)

where $x_i$ is the variable relating to the geographical and/or environmental conditions of the $i$th 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.
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 $0.247 \pm 0.079$, $0.197 \pm 0.052$ and $0.238 \pm 0.077$, 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. 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.
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  |
| Origin           | 13  | 106           | 5.7 ***  | 4.7     | 735           | 3.4 ***  | 2.4     | 0.068        | 23.2 *** |
| Site             | 2   | 823           | 18.0 *** | 17.4    | 9436          | 21.9 *** | 18.3    | 0.178        | 11.9 *** |
| Replication (site)| 6  | 41            | 2.2 *    | 1.1     | 402           | 1.9      | 0.9     | 0.019        | 6.5 ***  |
| Origin × site    | 24  | 40            | 2.2 ***  | 5.3     | 363           | 1.7 *    | 3.3     | 0.008        | 2.7 ***  |
| 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          | AIC | Shoot         | AIC | RMF          | 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 × 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).
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.

**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.
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.

Acknowledgments

The authors thank Dr. Shinji Fujii, Tropical Biosphere Research Center, University of the Ryukyus for the field survey, and Dr. Miki Ueda for the measurement of N contents. We also thank Dr. Nobuharu Fujii, Dr. Seikan Kurata, staffs of the University of Tokyo Forests, the University of Tokyo and staffs of the Institute of Genetic Ecology, Tohoku University for the assistance of the field cultivation tests, and anonymous reviewers for critical comments on the manuscript. This study was supported by JST CREST Grant Number JPMJCR11B3 Japan, JSPS KAKENHI Grant Number 24770014 and 15K06901 and Inamori Foundation.

Literature Cited

Andrés, F. and G. Coupland (2012) The genetic basis of flowering responses to seasonal cues. Nat. Rev. Genet. 13: 627–639.
Aoba, T. (1988) Pedigree and variation of wild radish in Japan. Technol. Agric. 12: 94–114.
Barrett, D.H.R. and D. Schluter (2008) Adaptation from standing genetic variation. Trends Ecol. Evol. (Amst.) 23: 38–44.
Byars, S.G., W. Papst and A.A. Hoffmann (2007) Local adaptation and cogradient selection in the alpine plant, Poa niemata, along a narrow altitudinal gradient. Evolution 61: 2925–2941.
Carputo, D., D. Alioto, R. Aversano, R. Garramone, V. Miraglia, C. Villano and L. Frusciano (2013) Genetic diversity among potato species as revealed by phenotypic resistances and SSR markers. Plant Genet. Resour. 11: 131–139.
Doane, T.A. and W.R. Horwáth (2003) Spectrophotometric determination of nitrate with a single reagent. Anal. Lett. 36: 2713–2722.
Dwivedi, S.L., S. Cecccarelli, M.W. Blair, H.D. Upadhyaya, A.K. Are and R. Ortiz (2016) Landrace germplasm for improving yield and abiotic stress adaptation. Trends Plant Sci. 21: 31–42.
Fitter, A.H. and R.K.M. Hay (1981) Environmental physiology of plants. Academic Press, New York, p. 355.
Fujieda, K. (1993) Radish. In: Fujieda, K. (ed.) Origin and divergence of vegetables, Univ. Kyusyu Press, Fukuoka, pp. 78–82.
Fujii, T. (1977) Radish. Asahi-Encyclopedia. World Plant 61: 1403–1315.
Han, Q., H. Higashi, Y. Mitsui and H. Setoguchi (2015) Distinct phyllogeographic structures of wild radish (Raphanus sativus L. var. raphanistroides Makino) in Japan. PLoS ONE 10: e0135132.
Han, Q., H. Higashi, Y. Mitsui and H. Setoguchi (2016) Lineage isolation in the face of active gene flow in the coastal plant wild radish is reinforced by differentially vernalised responses. BMC Evol. Biol. 16: 84.

Hikosaka, K., K. Ishikawa, A. Borjigidai, O. Muller and Y. Onoda (2006) Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. J. Exp. Bot. 57: 291–302.

Huh, M.K. and O. Ohnishi (2001) Allozyme diversity and population structure of Japanese and Korean populations of wild radish, Raphanus sativus var. hortensis f. raphanistroides (Brassicaceae). Genes Genet. Syst. 76: 15–23.

Ishizuka, W. and S. Goto (2012) Modeling intraspecific adaptation of Abies sachalinensis to local altitude and responses to global warming, based on a 36-year reciprocal transplant experiment. Evol. Appl. 5: 229–244.

Iwata, H., S. Niikura, S. Matsuura, Y. Takano and Y. Ukai (1998) Evaluation of variation of root shape of Japanese radish (Raphanus sativus L.) based on image analysis using elliptic Fourier descriptors. Euphytica 102: 143–149.

Johnson, J.B. and K.S. Omland (2004) Model selection in ecology and evolution. Trends Ecol. Evol. (Amst.) 19: 101–108.

Jonas, C.S. and M.A. Geber (1998) Variation among populations of Clarkia unguiculata (Onagraceae) along altitudinal and latitudinal gradients. Am. J. Bot. 86: 333–343.

Kaneko, Y., S.W. Bang and Y. Matsuzawa (2011) Raphanus. In: Kole, C. (ed.) Wild Crop Relatives: Genomic and Breeding Resources, Vegetables, Springer, Heidelberg, pp. 247–258.

Kang, E.S., S.M. Ha, H.C. Ko, H.J. Yu and W.B. Chae (2016) Reproductive traits and molecular evidence related to the global distribution of cultivated radish (Raphanus sativus L.). Plant Syst. Evol. 302: 1367–1380.

Katoh, A., H. Ashida, I. Kasajima, S. Shigeoka and A. Yokota (2015) Potato yield enhancement through intensification of sink and source performances. Breed. Sci. 65: 77–84.

Kausik, P., J. Prohens, S. Vilanova, P. Gramazio and M. Plazas (2016) Phenotyping of eggplant wild relatives and interspecific hybrids with conventional and phenomics descriptors provides insight for their potential utilization in breeding. Front. Plant Sci. 7: 677.

Kawecki, T.J. and D. Ebert (2004) Conceptual issues in local adaptation. Ecol. Lett. 7: 1225–1241.

Kim, N., Y.M. Jeong, G.B. Kim, S. Baek, Y.E. Kwon, A. Cho, S.B. Choi, J. Kim, W.J. Lim et al. (2016) Identification of candidate domestication regions in the radish genome based on high-depth resequencing analysis of 17 genotypes. Theor. Appl. Genet. 129: 1797–1814.

Kitamura, S. (1968) Phytogeography of Shiga Prefecture. In: Kitamura, S. (ed.) Flora of Shiga Prefecture, Hoikusha, Osaka, Japan, pp.1–20.

Körmer, C.H. and U. Renhardt (1987) Dry matter partitioning and root length/leaf area ratios in herbaceous perennial plants with diverse altitudinal distribution. Oecologia 74: 411–418.

Lammers, J.S., F.S. Chapin and T.L. Pons (2008) Plant physiological ecology. New York, Springer.

Li, B., J.I. Suzuki and T. Hará (1998) Latitudinal variation in plant size and relative growth rate in Arabidopsis thaliana. Oecologia 115: 293–301.

Lü, N., K. Yamane and O. Ohnishi (2008) Genetic diversity of cultivated and wild radish and phylogenetic relationships among Raphanus and Brassica species revealed by the analysis of trnK/matK sequence. Breed. Sci. 58: 15–22.

Mazer, S.J. and L.M. Wolfe (1992) Planting density influences the expression of genetic variation in seed mass in wild radish (Raphanus sativus L.: Brassicaceae). Am. J. Bot. 79: 1185–1193.

Mitsui, Y., M. Shimomura, K. Komatsu, N. Namiki, M. Shibata, M. Imai, Y. Katayose, Y. Mukai, H. Kanamori, K. Kurita et al. (2015) The radish genome and comprehensive gene expression profile of tuberous root formation and development. Sci. Rep. 5: 10835.

Nie, S., C. Li, L. Xu, Y. Wang, D. Huang, E.M. Muleke, X. Sun, Y. Xie and L. Liu (2016) De novo transcriptome analysis in radish (Raphanus sativus L.) and identification of critical genes involved in bolting and flowering. BMC Genomics 17: 389.

Oihaso, T., M. Hirai and M. Yamabuki (2010) Spatial structure of microsatellite variability within and among populations of wild radish Raphanus sativus L. var. hortensis Backer f. raphanistroides Makino (Brassicaceae) in Japan. Breed. Sci. 60: 195–202.

Oleskys, J., M.G. Tjoelker and P.B. Reich (1992) Growth and biomass partitioning of populations of European Pinus sylvestris L. under simulated 50° and 60°N daylengths: evidence for photo-periodic ecotypes. New Phytol. 120: 561–574.

Oleskys, J., J. Modrzyńska, M.G. Tjoelker, R. Zytkowski, P.B. Reich and P. Karolewski (1998) Growth and physiology of Picea abies populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. Funct. Ecol. 12: 573–590.

Olsson, K. and J. Ägren (2002) Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb Lythrum salicaria. J. Evol. Biol. 15: 983–996.

Poorter, H., K.J. Niklas, P.B. Reich, J. Oleksyn, P. Poot and L. Mommer (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193: 30–50.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reeves, T.G., G.R. Code and C.M. Piggim (1981) Seed production and longevity, seasonal emergence and phenology of Wild Radish (Raphanus raphanistrum). Aust. J. Exp. Agric. 21: 524–530.

Rehfelt, G.E., N.M. Tchekyakova, Y.I. Parfenova, W.R. Wykoff, N.A. Kuzmina and L.I. Miljutin (2002) Intraspecific responses to climate in Pinus sylvestris. Glob. Chang. Biol. 8: 912–929.

Ridley, C.E. and N.C. Ellstrand (2010) Rapid evolution of morphology and adaptive life history in the invasive California wild radish (Raphanus sativus) and the implications for management. Evol. Appl. 3: 64–76.

Schenk, H.J. and R.B. Jackson (2002) Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. J. Ecol. 90: 480–494.

Skroppa, T. and S. Magnusen (1993) Provenance variation in shoot growth components of Norway spruce. Silva Genet. 42: 111–120.

Stinchcombe, J.R., C. Weinig, M. Ungerer, K.M. Olsen, C. Mays, S.S. Hallidorsdottir, M.D. Purugganan and J. Schmitt (2004) A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. Proc. Natl. Acad. Sci. USA 101: 4712–4717.

Sugiuira, D., E. Betsuyaku and I. Terashima (2015) Manipulation of the hypocotyl sink activity by reciprocal grafting of two Raphanus sativus varieties: its effects on morphological and physiological traits of source leaves and whole plant growth. Plant Cell Environ. 38: 2629–2640.
Wang, N., N. Kitamoto, R. Ohsawa and T. Fujimura (2008) Genetic diversity of radish (*Raphanus sativus*) germplasms and relationships among worldwide accessions analyzed with AFLP markers. Breed. Sci. 58: 107–112.

Warwick, S.I., A.L.A. Francis and R.K. Gugel (2009) Guide to wild germplasm of Brassica and allied crops (tribe *Brassiceae*, Brassicaceae), 3rd edn. Agriculture and Agri-Food Canada Research Branch Publication, Ottawa, online: http://www.brassica.info/info/publications/guide-wild-germplasm.php.

Weber, E. and B. Schmid (1998) Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. Am. J. Bot. 85: 1110.

Yamaguchi, H. (1987) Latitudinal cline and intrapopulational differentiation in leaf shape of wild radish in Japan. Japan. J. Breed. 37: 54–65.

Yamane, K., N. Lü and O. Ohnishi (2009) Multiple origins and high genetic diversity of cultivated radish inferred from polymorphism in chloroplast simple sequence repeats. Breed. Sci. 59: 55–65.

Yoshida, Y., N. Takada and Y. Koda (2010) Isolation and identification of an anti-bolting compound, hexadecatrienoic acid monoglyceride, responsible for inhibition of bolting and maintenance of the leaf rosette in radish plants. Plant Cell Physiol. 51: 1341–1349.

Yoshie, F. (2007) Length of the pre-reproductive period of *Plantago asiatica* L. from different latitudes. Plant Species Biol. 22: 135–139.