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The effect of plant architecture on drought resistance: implications for the evolution of semelparity in *Erysimum capitatum*

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Summary

1. Constraints of resource allocation between reproduction and adult survival have been implicated in much life-history variation, yet physiological or functional trade-offs with juvenile survival may be just as important. Here, we examined selection on a juvenile trait that is a key determinant of semelparous (monocarpic) vs. iteroparous (polycarpic) life-history expression.

2. In *Erysimum capitatum*, iteroparous plants produce more rosettes at the juvenile stage than do semelparous plants; those rosettes perennate, enabling subsequent reproductive episodes. Thus, the number of rosettes produced before reproduction is a strong determinant of iteroparity. We tested whether increased rosette production compromised juvenile survival under conditions similar to those in which semelparity predominates over iteroparity.

3. Using plants from six natural populations, we tested the association between rosette production and juvenile survival under drought conditions typical of the field sites of semelparous *E. capitatum* populations. We also manipulated rosette number by physically removing rosettes and examined the effect of rosette removal on drought resistance.

4. Under drought conditions, plants with fewer rosettes had higher survival, and the physical excision of rosettes improved survival (significantly or marginally) under drought stress in five of six natural populations.

5. The lower production of rosettes, typical of semelparous *E. capitatum*, was associated with increased juvenile survival under drought stress. The results suggest adaptive differentiation of rosette production, at least partially in response to drought stress. Given the role of apical dominance in multiple rosette development, natural selection seems to favour stronger apical dominance under drought conditions. Drought stress is predicted to be more common at high elevation as a result of climate change, and the novel drought stress could increase juvenile mortality of alpine *E. capitatum*. Because rosette production at the juvenile stage is necessary for iteroparity, these results demonstrate that drought-induced selection on traits that determine early survival has significant potential to influence the evolution of adult life-history expression.

Key-words: adaptive differentiation, drought resistance, life history, monocarpy, natural selection, polycarpy, rosette development

Introduction

Adult survival is considered to be a major factor determining the evolution of reproductive schedules. High adult mortality has been implicated in the evolution of early and condensed reproductive schedules in a number of species, including vertebrates, insects and plants (Harvey & Zammuto 1985; Franco & Silvertown 1996; Reznick *et al.* 1996; Dudycha & Tessier 1999; Stearns *et al.* 2000; Lesica & Young 2005). Resource trade-offs between adult survival and reproduction may exist, such that prior reproduction compromises subsequent survival and reproduction, and they have been the focus of many studies of the evolution of reproductive strategies (Williams 1966; Schaffer & Rosenzweig 1977; Reznick 1985; Obeso 2002). Functional trade-offs at the adult stage may also be important determinants

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of reproductive timing (Price & Schluter 1991; Endler 1995), as demonstrated in sticklebacks, in which high fecundity during a single reproductive bout compromised swimming performance, thereby favouring smaller, more frequent reproductive bouts (Foster, Baker & Bell 1992; Ghalambor, Reznick & Walker 2004).

However, selection also occurs at juvenile stages. As such selection necessarily occurs before selection at adult stages, and as organisms must survive early stages to express adaptive traits at later stages, adaptation of juvenile stages is hypothesized to be especially important in certain contexts, especially during colonization, range expansion or changing environments (Schluter, Price & Rowe 1991; Stratton 1992; Maun 1994; Miriti 2006; Poorter 2007; Donohue *et al.* 2010; Huang *et al.* 2010). If certain physiological or developmental traits influence both early survival and adult reproduction, natural selection acting on those traits at the juvenile stage would also affect the evolutionary dynamics of adult reproductive strategies (Lande 1982; Partridge *et al.* 1991). Thus, the developmental context of life-history variation must be evaluated to predict how selection across the entire life cycle will influence life-history evolution.

Semelparity is a widespread reproductive strategy in both animal and plant kingdoms. Semelparous (monocarpic) organisms have a single reproductive episode followed by the death of the organism, while iteroparous (polycarpic) organisms reproduce repeatedly throughout their lifetime (Young & Augspurger 1991; Roff 1992; Stearns 1992). A major hypothesis regarding the evolution of semelparity is that, when adult mortality is high compared with juvenile mortality, a semelparous genotype with a higher reproductive output would have greater fitness than an iteroparous genotype with a lower reproductive output per reproductive episode (Schaffer & Rosenzweig 1977; Young 1981; Orzack & Tuljapurkar 1989).

Interestingly, variation of semelparity/iteroparity in plant species is often associated with their architecture. For instance, many long-lived semelparous plant taxa form a single apical rosette, while closely related iteroparous taxa produce multiple rosettes and/or branches from the apical rosette (Young & Augspurger 1991). In iteroparous species, only a subset of rosettes contributes to a particular reproductive episode by developing determinate inflorescences; nonreproductive rosettes remain vegetative during reproduction and are able to produce inflorescences in future growing seasons. In contrast, semelparous plants frequently produce indeterminate inflorescences from their single apical rosette, and vegetative tissues of the apical rosette degenerate during reproduction. Notably, rosettes that are necessary for iteroparity develop at the juvenile stage. Therefore, at least for some rosette plant species, the production of additional vegetative rosettes at the juvenile stage is likely to be a developmental prerequisite for an iteroparous strategy as an adult (Silvertown 1989).

In such species, natural selection on rosette production at the juvenile stage has the potential to influence the evolution of parity expressed at the adult stage. A more general question concerning the evolution of semelparity/iteroparity is whether the morphological traits required for iteroparous reproduction are subjected to natural selection at earlier life stages. If juvenile traits are prerequisites for adult reproductive strategies, then selection on those traits at the prereproductive stage may influence the evolution of adult life histories.

Western wallflowers (*Erysimum capitatum,* Brassicaceae) in the Colorado Rocky Mountains exhibit altitudinal variation in iteroparity (Price 1987), and such variation is manifest in a common greenhouse environment (Table 1). *Erysimum capitatum*, especially those from high-elevation populations, produce multiple rosettes at the axils of leaves on the apical rosette (i.e. axillary rosettes; Fig. 1). Notably, in both field and greenhouse environments, plants from low-elevation semelparous populations produced significantly fewer rosettes at the prereproductive stage than plants from high-elevation iteroparous environments (Kim & Donohue 2011a). In addition, plants with more rosettes at the prereproductive stage had more vegetative rosettes after reproduction, which in turn positively influenced survival after reproduction and the opportunity for future reproductive episodes (Kim & Donohue 2011b). Thus, in *E. capitatum*, production of multiple rosettes at the juvenile stage is a morphological prerequisite for iteroparity.

Not only are populations of *E. capitatum* differentiated across altitude in rosette production and iteroparity, but plants from low elevation have higher fitness at low elevation than plants from high elevation (E. Kim, unpublished data). When plants from high-elevation populations were transplanted into low-elevation sites, they produced more rosettes and suffered higher mortality during the summer than those from low-elevation populations. Water availability at the low-elevation site was extremely low during the summer (Kim & Donohue 2011a), suggesting drought may have imposed selection on rosette production, resulting in lower survival of high-elevation populations at low-elevation sites. Here, we investigated selection on rosette production at the juvenile stage in the western wallflower under different conditions of water availability.

First, we tested whether natural variation in rosette production was associated with juvenile survival under low-water conditions (drought stress) in the greenhouse. We then manipulated the number of rosettes by physically removing rosettes to examine whether rosette number influenced survival under drought conditions. Specifically, the following questions were addressed: (i) Do populations from different altitudes show differential survivorship in drought conditions in the greenhouse? (ii) Is the production of axillary rosettes associated with survivorship under drought stress? (iii) Does the removal of axillary rosettes improve survivorship under drought stress? Given that plants living in drought conditions have fewer rosettes (and are more semelparous) than those living in nondrought conditions, we predicted that plants with fewer rosettes would have higher juvenile survival under drought stress, but not under conditions of abundant water.

Table 1. Source populations of *Erysimum capitatum*, with their elevation and life history observed *in situ* and in a greenhouse environment with benign soil water conditions

Region	Population	Elevation (m a.s.l.)	Life history	Post-reproductive survival Field	Post-reproductive survival Greenhouse
1 (Gunnison county, CO)	H1	3191	Iteroparous	86·9 (2·4)	84·1 (5·5)
	L1	2630	Intermediate	17·0 (9·4)	63·4 (7·5)
2 (Clear Creek county, CO)	H2	3636	Iteroparous	71·8 (11·7)	90·0 (4·7)
	L2	2234	Semelparous	0·0 (0·0)	53·3 (7·4)
3 (Boulder county, CO)	H3	3505	Intermediate	20·1 (9·4)	76·3 (6·9)
	L3	1831	Semelparous	3·5 (3·5)	51·2 (7·8)

Survival rate of reproducing plants in the field environments were measured from 2004 to 2007. Average (SE) of postreproductive survival rate is given.

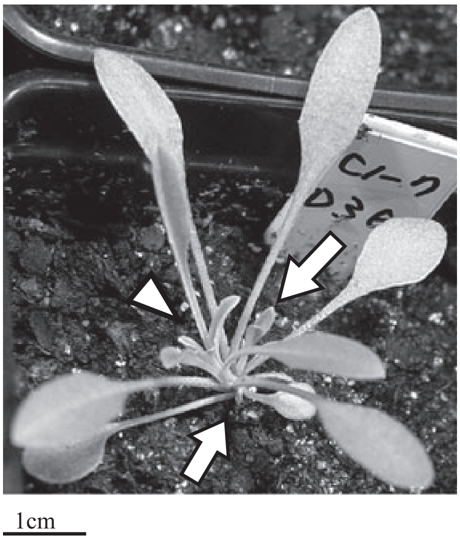

Fig. 1. *Erysimum capitatum* grown in a greenhouse environment. A seed from the H2 population was germinated and grown for 4 weeks in high water condition in a greenhouse environment described in the Materials and Methods. Triangle indicates the centre of apical rosette, and arrows indicate axillary rosettes developed from axils of leaves on the apical rosette. The scale bar represents 1 cm.

Materials and methods

STUDY POPULATIONS AND SOIL WATER ENVIRONMENTS

Natural populations of *E. capitatum* were identified in three alpine tundra environments (H1, H2 and H3) (altitude > 3260 m a.s.l.) and in three lower-elevation environments (L1, L2 and L3) (altitude < 2640 m a.s.l.) on three separate watersheds in Colorado, USA (Table 1). In this study, 'high-elevation populations' refers to plants from alpine tundra environments, 'low-elevation populations' refers to those from lower-elevation environments, and 'region' refers to each watershed. Natural variation in iteroparity was found among these six populations, such that the H1 and H2 populations are unambiguously iteroparous, the L2 and L3 are unambiguously semelparous and the H3 and L1 populations exhibited an intermediate between those two extremes (Kim & Donohue 2011a; Table 1). Soil water content of semelparous L2 and L3 populations was significantly lower than other populations in 2007, and mean soil moisture measured in August 2007 positively correlated with the mean postreproductive survival of the source populations (Kim & Donohue 2011a).

EXPERIMENTAL DESIGN

Seeds from 15 field plants (maternal genotypes) of each population were sprinkled into flats filled with MetroMix 360 (Sungro Inc., Vancouver, BC, Canada) and saturated with water in the Harvard University greenhouse. Seedlings with four to six leaves were transplanted into 7·6 × 7·6 × 14 centimetre plastic pots with MetroMix 360 (Sungro Inc.). All plants were maintained at 17 °C with a 12-h photoperiod. Supplemental light from high-intensity mercury halide lights was provided between 6:00 am and 6:00 pm if ambient light fell below 760 μmol m^{-2} s^{-1} for more than 15 min. One individual per maternal genotype was randomly assigned to each of three 'predrought' treatments and two 'drought' conditions, resulting in a total of 540 plants (six populations × 15 maternal genotypes per population × three predrought treatments × two drought treatments). These plants were grown in three blocks, with seeds from five field plants per population in each block and experimental treatments randomly assigned within each block.

After the seedlings were transplanted, three 'predrought' treatments were applied to manipulate plant morphology before drought stress: fluctuating water (FW), high water (HW) and high water with rosette removal (HW-Cut). *Erysimum capitatum* plants in moister soil produce larger apical rosette and more axillary rosettes (Kim & Donohue 2011b). Two water levels were imposed to plants at the age of 20–23 days to induce differences in rosette production: a fluctuating water treatment [FW, 10–30% (v/v)] that resembled natural fluctuations in water conditions in the field, and a high water treatment (HW, 20–30% (v/v)), in which more and larger rosettes would be produced. Predrought water treatments were applied for 1 month.

To test whether the presence of multiple rosettes itself influences survivorship under drought stress, an excision treatment was applied to plants that were grown under HW conditions (HW-Cut) at the age of 35–38 days. In the HW-Cut treatment, all rosettes except the apical rosettes were excised using small scissors 2 weeks before drought stress was applied. As physical damage like the excision of rosettes could cause physiological changes of plants, some leaf damage was applied to all nonexcised intact plants by cutting off half of the third smallest, fully expanded leaf. Cutting treatment did not have any effect on the maximum diameter of rosettes (Table 2).

A drought condition was applied to juvenile plants after predrought treatments. In the L2 and L3 *E. capiatum* habitats during the growing seasons in 2006 and 2007, soil water content was below 10% (v/v), and juvenile mortality was high (Kim & Donohue 2011a).

Table 2. Results of analysis of variance to compare survival and morphology between elevations of the source populations and between predrought treatments before drought stress

Traits	Elev (d.f. = 1)	Trt (d.f. = 1)	Elev × Trt (d.f. = 1)	Reg (d.f. = 2)	Elev × Reg (d.f. = 2)	Trt × Reg (d.f. = 2)	Elev × Trt × Reg (d.f. = 2)
(a) HW and FW treatments							
Survival	4·61*	2·03	0·33	0·08	0·94	3·58	3·36
Mean leaf number per rosette	1·88	0·77	2·38‡	0·62	0·41‡	1·67‡	0·02
Maximum diameter of rosettes	40·04***	33·39***	0·27	22·39***‡	0·28	1·31	1·64
Number of rosettes	35·77***	9·83**‡	0·01	2·52†‡	0·65	0·96	0·53
(b) HW and HW-cut treatments							
Survival	2·37	0·93	1·62	1·67	2·17	7·88*	5·5†
Maximum diameter of rosettes	25·63***	0·62	0·15	18·90***	0·24	0·39	0·27

Plants from the nondrought condition were not included in the analysis because of lack of variance of plant survival. Chi-square values are given for survival, and F values are given for morphological traits. The mean leaf number per rosette was natural-log transformed to meet normality assumptions. The maximum diameter of rosettes was compared between HW and HW-Cut treatments, but the number of rosettes and leaf number per rosette were not analysed because axillary rosettes were removed.

†P < 0·1, *P < 0·05, **P < 0·01, ***P < 0·001. Elev, elevation; Trt, predrought treatment; Reg, region.

‡Significance differs between fixed and mixed models with region and its interaction with other factors as random factors.

Therefore, the drought stress was applied to half the plants by maintaining soil water content below 10% (v/v).

To implement the water manipulations, Metro-Mix 360 (Sungro Inc.) was air-dried for 10 days, and the same amount of soil was placed in each pot. Volumetric water content (v/v), which was measured in natural populations, was converted into gravimetric water content (w/w) based on soil density (Campbell Scientific Inc., 2001). The soil water content of each pot was monitored by weighing the pots every 3 days, and water was added as necessary to achieve the highest weight of water treatments every 3 days for HW, every 6 days for FW and every 3 days for 'drought stress'. Measurement of pot weights showed that each pot in drought stress needed 30 mL of water every week to maintain soil water content of 5–10%, so 30 mL of water was added every week. The other half of the plants, in the nondrought treatment, were watered with 100 mL of water every week.

At the beginning of the drought stress, the number of rosettes, the diameter and number of leaves of all rosettes on each plant were recorded. As an indicator of plant size, the mean leaf number per rosette was calculated as the number of leaves of an individual plant divided by the number of rosettes. The mean leaf number per rosette, the maximum diameter of rosettes and the number of rosettes were used for statistical analyses. Every 2 weeks, plants with no viable green leaves were scored as dead. Six months after the start of the drought treatment, when more than half of the plants under drought stress had no viable leaves, 100 mL of water was added to all pots every week, and the final mortality was determined after 1 month of watering.

The same morphological traits were measured when the HW-cut treatment was applied (i.e. 15 days after predrought water treatments started to be applied). Plants had a similar number of rosettes and a similar mean leaf number per rosette across predrought treatments and drought conditions. However, plants that were assigned to the HW predrought treatment had rosettes with around 2 mm larger maximum diameters compared with those assigned to the FW treatment (See Table S1, Supporting Information for statistical analysis). On average, plants from high-elevation populations had 3·0 ± 0·1 (SE) rosettes with 6·6 ± 0·2 leaves per rosette and 29·81 ± 0·44 mm maximum diameter, and those from low-elevation populations had 2·3 ± 0·1 rosettes with 8·1 ± 0·2 leaves per rosette and 29·19 ± 0·43 mm maximum diameter.

In nondrought conditions, some plants produced flowers during the experiment (Seven H1 plants, 19 H2 plants, 15 H3 plants and 11 L1 plants). However, no flowering was observed in the drought condition. In this experiment, we focused on prereproductive (juvenile) mortality in order not to confound vegetative mortality with postreproductive mortality.

STATISTICAL ANALYSES

All statistical analyses used the SAS statistical package ver. 9·2. Separate analyses were conducted on intact plants (HW vs. FW), and within same water treatment (HW vs. HW-Cut).

To compare mortality between drought and nondrought treatments, analysis of variance was conducted. A logistic model with a binomial error distribution was constructed, with binary survival that was determined at the end of experiment (One for surviving plants and zero for dead plants) as the dependent variable. Predrought treatment, drought treatment, source elevation of populations (high- vs. low-elevation), region and block were independent factors (SAS Proc GENMOD). Interactions among independent variables could not be included in the model because the model that included interactions would not converge owing to lack of variance in the nondrought treatment, as only four plants died in the nondrought treatment (Allison 1999). χ^2 values from likelihood ratio tests were calculated to evaluate the significance of the independent variables.

Subsequent analyses were conducted only on plants under drought conditions because a lack of variance in mortality of nondroughted plants precluded further analysis of the nondrought treatment. To test whether survival under drought differed between high- and low-elevation populations and across predrought treatments (HW vs. FW or HW vs. HW-Cut), analysis of variance was conducted. A logistic model with a binomial error distribution was constructed including binary survival at the end of experiment as the dependent variable and predrought treatment, elevation, region and their interactions as independent fixed factors (SAS Proc GENMOD). A block term was also included in all models to control for microenvironmental effects on survivorship. As there was a significant interaction between predrought treatment and region (see Results), additional analyses of variance were conducted to determine the treatment effect on survival within each population separately.

To test whether predrought juvenile morphological traits differed between high- and low-elevation populations, and between predrought water treatments (HW and FW), analyses of variance (SAS Proc GLM) were conducted using the same model as that used for survival. The mean leaf number per rosette, the maximum diameter of rosettes and the number of rosettes were compared. The mean leaf number per rosette was natural-log transformed to meet normality assumptions. Mixed model analyses of variance with region as a random factor were also conducted, and the results were qualitatively similar to fixed model analyses of variance. The maximum diameter of rosettes was also compared between HW and HW-Cut treatments using the same model. Number of rosettes and leaf number per rosette were not analysed because axillary rosettes were removed in HW-Cut treatment.

Logistic regression analysis was conducted to test for effects of juvenile morphological traits on survival under drought stress in intact plants (HW and FW treatments) (SAS Proc Logistic). The population and treatment terms were included to control for possible differences between populations and treatments in unmeasured traits (including drought acclimation, independent of morphological response to drought). A block term was also included in all models. Survival at the end of the experiment was the dependent variable, and the mean leaf number per rosette, the maximum diameter of rosettes and the number of rosettes were independent variables. The 'Total effect' of each trait was evaluated by conducting a simple logistic regression including one trait in the model, and the 'Direct effect' of trait was tested using multiple logistic regression. Both linear and quadratic coefficients were evaluated. All dependent variables were standardized with zero mean and one SD. To test for differences in regression coefficients between source elevation and between predrought water treatments, analysis of covariance was conducted. Interactions between morphological traits and elevation or between morphological traits and predrought water treatment would indicate that effects of the morphological traits on survival differed among source elevations or between predrought water treatments.

To determine the contribution of traits to the observed elevational differentiation in survival of intact plants under drought stress, analysis of covariance was performed. The same model that was used to test for elevational differentiation in survival was used with the traits as covariates, and the effect of elevation in this model was compared with that in which trait covariates were not included.

Results

SURVIVAL RESPONSE TO DROUGHT STRESS

All plants except four (98·52%) survived until the end of experiment in the nondrought treatment (one H3 plant in FW, one L3 plants in HW and two H1 plants in HW-Cut), compared with only 34·1% survival in drought-stressed plants, averaged across all predrought treatments (Fig. 2). Thus, plant death in the 5–10% water condition was likely due to drought stress [χ^2(drought) for FW and HW treatment = 236·31, $P < 0·001$; χ^2 (drought) for HW and HW-Cut treatment = 185·50, $P < 0·001$]. Such high survival in the nondrought condition implies that there is no source elevation effect on survival and no correlation between morphological traits and survival in a benign water conditions, even though the data could not be analysed with a logistic model owing to lack of variance.

POPULATION DIFFERENTIATION AND PLASTICITY OF INTACT PLANTS

Both source elevation and predrought water treatment affected juvenile morphological traits (Table 2, Fig. 3). Plants from low-elevation populations produced larger but fewer rosettes compared with those from high-elevation populations. In addition, plants that were grown in wetter conditions had larger and more (marginally significant in a mixed model) rosettes. No source-elevation effect or predrought water-treatment effect was found for the mean leaf number per rosette, although plants from low elevation had slightly more leaves per rosette in FW than HW, based on a mixed model (Table 2).

Plants from high-elevation populations survived less under drought conditions than did those from low-elevation populations (Table 2, Fig. 2). Water treatment before drought stress did not influence the tolerance to drought stress, as indicated by the nonsignificant effect of water treatment and its interaction with elevation or region. Thus, acclimation to low-water availability before drought (FW) did not increase survival.

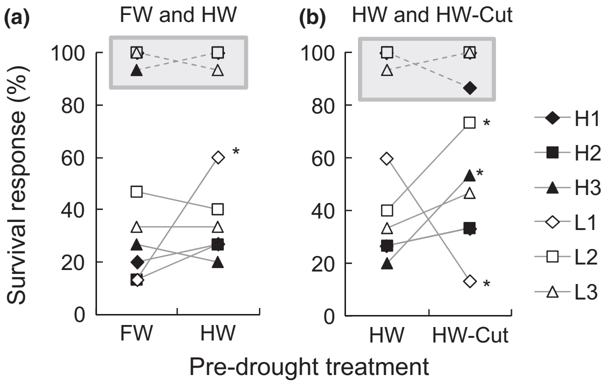

Fig. 2. Differences in survival across predrought treatments. Asterisks indicate the significance level of the difference between treatments for each population. Survival in the nondrought condition is also shown within grey box. Symbols representing survivorship of the H1 and H2 populations in HW and HW-Cut predrought treatments under drought condition were overlapped since survivorship of those populations were same. FW = fluctuating water treatment, HW = high water treatment, HW-Cut = high water with cutting treatment. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

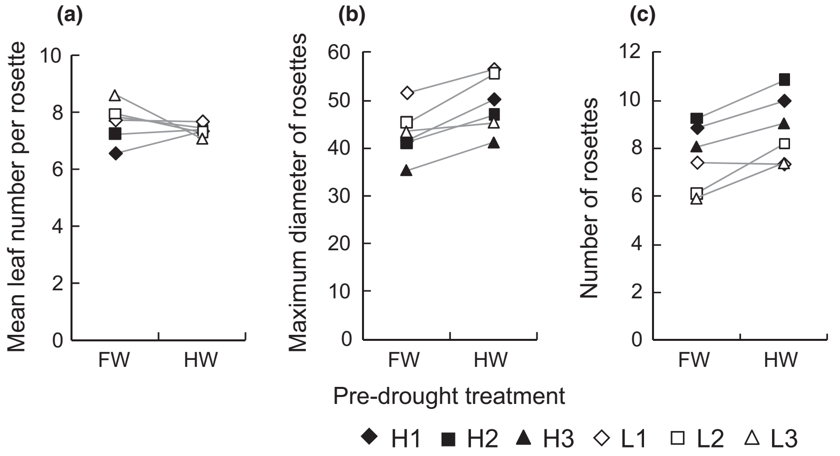

Fig. 3. Elevational differentiation in morphological traits and their plastic responses to predrought water treatment. Unadjusted means are shown. Error bars are not presented for clarity. HW = high water treatment, FW = fluctuating water treatment.

EFFECTS OF MORPHOLOGICAL TRAITS ON SURVIVAL UNDER DROUGHT STRESS

Logistic regression analysis indicated that juvenile morphological traits influenced the survival of intact plants under drought stress (Table 3). Under drought stress, the multiple regression analysis revealed direct effects of the mean leaf number per rosette and the number of rosettes, such that plants with fewer leaves per rosette and fewer rosettes survived more under drought stress. However, the total effects of the morphological traits were not significant, indicating that correlations among traits cancel out their net effects on survival (Table 3).

No significant effect of the trait × elevation interaction on survival was detected in drought stress, indicating that effects of morphological traits on survival under drought stress are consistent in both high- and low-elevation populations ($\chi^2 < 1{\cdot}31$, $P > 0{\cdot}25$ in all cases). The direct effect of leaf number per rosette differed marginally across predrought water treatments ($\chi^2 = 3{\cdot}53$, $P < 0{\cdot}1$), but direct effects of the other traits did not differ between predrought water treatments ($\chi^2 < 0{\cdot}21$, $P > 0{\cdot}65$).

Quadratic coefficients were nonsignificant, but the correlational coefficient of the (maximum diameter of rosettes) × (the number of rosettes) differed between water treatments ($\chi^2 = 6{\cdot}17$, $P < 0{\cdot}05$). The coefficient was positive with marginal significance in the FW treatment (coefficient = 0·81, SE = 0·42, $P = 0{\cdot}057$), such that plants with fewer and smaller rosettes (or more and larger rosettes) had higher survival, but not plants with fewer and larger rosettes (or more but smaller rosettes). The correlational coefficient was not significant in the HW treatment (coefficient = −0·36, SE = 0·34, $P = 0{\cdot}29$).

Differences in morphology across elevation accounted for the differential survival of the populations under drought stress (Table 4). Inclusion of maximum diameter of rosettes and number of rosettes, but not mean leaf number per rosette, caused differential survival between elevations to become nonsignificant.

EFFECT OF REMOVAL OF AXILLARY ROSETTES ON THE SURVIVAL RESPONSE TO DROUGHT STRESS

The main effect of the cutting treatment on survival was not significant, but a significant treatment × region interaction and a marginally significant elevation × treatment × region interaction were detected (Table 2), indicating that the effect

Table 3. Effects of morphological traits on survivorship under drought stress

	Regression coefficients			
	Linear		Nonlinear	
	T (SE)	*D* (SE)	*T* (SE)	*D* (SE)
Mean leaf number per rosette	−0·23 (0·20)	−0·57* (0·27)	−0·14 (0·16)	−0·21 (0·36)
Maximum diameter of rosettes	0·45 (0·23)†	0·45 (0·24)†	0·12 (0·16)	0·15 (0·16)
Number of rosettes	−0·23 (0·20)	−0·63* (0·27)	−0·14 (0·15)	0·28 (0·28)

Simple (Total = '*T*') and multiple (Direct = '*D*') logistic regression coefficients (SE) of standardized variables on survival of HW and FW plants at the end of experiment are shown. Coefficients of both linear and quadratic terms were estimated. All models included block, source population, and predrought water treatment as factors.
†$P < 0{\cdot}1$, *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

Table 4. Result of analysis of covariance to determine the contribution of morphological traits to the differential survivorship of high- and low-elevation populations under drought stress

	χ^2 (Elevation)
No trait	4·61*
All traits	0·22
Mean leaf number per rosette	5·47*
Maximum diameter of rosettes	1·38
Number of rosettes	2·67

The dataset with intact plants (FW and HW) was tested. Full models including block, elevation, and predrought water treatment were tested with or without morphological traits. χ^2 for elevation of source population is given. See Table S2 (Supporting Information) for the complete output of each model.
$*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

of the cutting treatment differed among populations. Cutting of axillary rosettes significantly increased drought tolerance in the H3 ($\chi^2 = 3{\cdot}98$, $P < 0{\cdot}05$) and the L2 ($\chi^2 = 4{\cdot}61$, $P < 0{\cdot}05$) populations (Fig. 2). Survivorship of plants with a cutting treatment was also higher in H1, H2 and L3 populations even though the difference in survivorship was not significant (H1: $\chi^2 = 0{\cdot}16$, $P > 0{\cdot}1$, H2: $\chi^2 = 0{\cdot}18$, $P > 0{\cdot}1$, L3: $\chi^2 = 0{\cdot}75$, $P > 0{\cdot}38$). The only population that did not show any increase in survival with cutting was the L1 population. When this population was dropped from the analysis, the main effect of the cutting treatment was significant ($\chi^2 = 5{\cdot}26$, $P < 0{\cdot}05$). The effect of cutting was apparent only under drought stress; only one intact plant in HW and two plants in HW-Cut died at the end of experiment when plants were maintained in nondrought condition.

Discussion

Erysimum capitatum from high-elevation populations suffered higher mortality under drought stress than those from low-elevation populations, and such differential survival was accounted for by differences in rosette size and number. Plants with fewer rosettes survived more under drought stress, and the physical excision of rosettes improved survival under drought stress, significantly in two populations and nonsignificantly in three others. Therefore, low soil water availability in the field likely favours the production of fewer rosettes at the juvenile stage. As production of multiple rosettes at the juvenile stage promotes postreproductive survival and enables an iteroparous life history, this experiment suggests that natural selection against the production of rosettes at the juvenile stage under drought conditions likely contributes to the evolution of a semelparous reproductive strategy in dry, low-elevation sites.

THE EFFECT OF THE AXILLARY ROSETTES ON SURVIVAL UNDER DROUGHT STRESS

A previous study demonstrated that when high-elevation plants were transplanted to low-elevation sites, they suffered higher mortality compared with those from native low-elevation populations (E. Kim and K. Donohue, unpublished data). Water availability was lower at low elevation, and much mortality occurred during the summer months when water availability was lowest. However, diverse environmental factors change along altitudinal gradients (Körner 2007), so the cause of the observed differential mortality was unclear. This experiment showed that high-elevation populations had lower survival under conditions of drought stress but not under conditions of abundant water. This result provides evidence that one major factor that contributes to differential survival along this altitudinal gradient is low-water availability at low-elevation sites.

Low-elevation populations also produced fewer axillary rosettes. Under drought conditions in this experiment, plants with fewer axillary rosettes were more likely to survive. The suppression of axillary rosette production is often accomplished by apical dominance (Mcsteen & Leyser 2005; Schmitz & Theres 2005). Various selective advantages of apical dominance have been suggested (Bonser & Geber 2005; Bonser & Aarssen 2006). For example, in herbaceous aclonal plants, strong apical dominance with less branch production is associated with a greater ability to compete for light, more effective pollinator attraction and greater protection of viable meristems from herbivory (Aarssen 1995). This experiment suggests another selective advantage of apical dominance: drought resistance.

Plants in dry environments exhibit diverse adaptations for water conservation or drought tolerance, including higher water use efficiency, higher concentration of specific macromolecules like proline as osmotic regulators, as well as changes in cell wall structures (Lambers, Pons & Chapin 2008; Mckay *et al.* 2009). A common adaptation to drought is to decrease leaf area by producing smaller leaves, producing fewer leaves or shedding of older leaves to minimize water loss (Chaves, Maroco & Pereira 2003). In this experiment, *E. capitatum* with fewer rosettes, and therefore lower total leaf area, survived longer, and an experimental reduction of the number of rosettes improved survival in drought conditions but had no effect under nondrought conditions. These results indicate that water loss through leaves of axillary rosettes could compromise survival under drought conditions. As plants close stomata in response to dry conditions, carbon starvation owing to long-term drought is another drought-related mortality factor (Mcdowell *et al.* 2008). Because young leaves or new meristems act as energy sinks (Vuorissalo & Mutikainen 1999), having a small number of large leaves in dry environments might be energetically more successful for growth compared with adding more rosettes with younger, more energetically expensive leaves. More study is required to understand the precise physiological mechanisms whereby the production of rosettes decreases drought resistance.

Three observations combined suggest that reduced rosette production has contributed to drought adaptation of low-elevation populations: the higher survival of plants from low elevation under drought conditions; the production of fewer rosettes by low-elevation plants; the increased survival of plants with fewer rosettes under drought. Although the presence of natural variation for the effect of rosette removal on drought tolerance suggests that different populations have evolved different mechanisms of drought tolerance, rosette production appears to be a significant trait involved in adaptive differentiation across altitude in *E. capitatum*.

This experiment documented both elevational differentiation and phenotypic plasticity of rosette number (see also Kim & Donohue 2011b). Alpine plants, although they had more rosettes than low-elevation plants, produced fewer rosettes in FW than HW, suggesting the potential for an adaptive plastic reduction in the number of rosettes. However, this plastic response did not significantly contribute to survival under drought stress in this experiment, even though the results of the multivariate analysis suggest a positive effect of reduced rosette production on the survival under drought stress. It should be noted that the positive effect of decreased rosette production on survival was significant only when the effects of other morphological traits were controlled for; plasticity of correlated traits apparently obscure the association of reduced rosette production on survival.

In response to climate change, environmental conditions at high elevation are projected to become similar to those at low elevation (Parry *et al.* 2007). In particular, increased temperature could cause earlier snowmelt and a longer growing season, which could also increase the evaporation and transpiration of soil water. As a consequence, a novel drought stress to plants might occur in alpine environments (Taylor & Seastedt 1994; Harte *et al.* 1995; Calanca 2007). This experiment showed that acclimation to low soil water content during FW predrought treatment did not improve survival of alpine *E. capitatum* under drought stress. In addition, apparent drought-induced juvenile mortality was higher in high-elevation than low-elevation plants when they were grown at low elevation (E. Kim, unpublished data). Thus, in *E. capitatum*, plants in alpine environments are expected to have higher mortality if a novel drought stress were to occur and stronger selection on traits that confer higher drought resistance. Adaptive plasticity or evolutionary responses to changed soil water content would be required to maintain populations in alpine environments (Kim & Donohue 2011b). This study suggests that adaptation to drought conditions, expected during climate change, has the potential to alter the fundamental life history of this plant.

IMPLICATIONS OF SELECTION ON JUVENILE TRAITS FOR ADULT LIFE-HISTORY EVOLUTION

In semelparous organisms, reproduction is followed by rapid senescence and death. This phenomenon has provoked much discussion concerning the selective factors that could possibly favour accelerated death (Cole 1954; Young & Augspurger 1991; Stearns 1992). A major hypothesis is that death of semelparous organisms is the result of antagonistic pleiotropy caused by resource-allocation trade-offs (Gadgil & Bossert 1970; Schaffer & Rosenzweig 1977). In this scenario, semelparous organisms invest all available resources in reproductive effort instead of in the maintenance of somatic viability, which results in death after a single-massive reproductive episode. According to this hypothesis, natural selection favours semelparity when increased reproductive effort compensates for reduced adult growth and/or survivorship, and the death of semelparous organisms is a by-product of natural selection for ever-increasing reproductive effort. Thus, allocational trade-offs between adult survival vs. reproduction can promote a semelparous life history. Theory also suggests that such extreme resource allocation as observed in semelparous organisms is the optimal life-history strategy when adult mortality is extrinsically high, and it is further influenced by population structure, environmental heterogeneity and density-dependent mortality (Orzack & Tuljapurkar 1989; Takada 1995; Ranta *et al.* 2000). High adult mortality and trade-offs between adult survival and reproduction are therefore central to theoretical formulations of the evolution of adult reproductive strategies.

This experiment shows that juvenile mortality and selection at the juvenile stage can also influence the evolution of adult reproductive strategies. Indeed, because organisms need to survive the juvenile stage before they can express any adult phenotype, selection on juveniles may act as a sieve that limits the phenotypes that can be expressed as adults, given correlations between juvenile and adult traits. In the case of *E. capitatum*, rosette production affects both juvenile drought resistance and the capacity for iteroparity. Specifically, the production of multiple rosettes at the juvenile stage is a prerequisite for an iteroparous strategy, as it provides perrenating tissue after reproduction that can contribute to subsequent reproductive episodes, yet it also decreases juvenile survival under drought conditions. This antagonistic pleiotropy, caused by trade-offs between physiological tolerances and developmental prerequisites for iteroparous reproduction, causes a negative association between juvenile survival and adult postreproductive survival. This contrasts with antagonistic pleiotropy via resource allocation, which causes trade-offs between early reproduction and adult viability. Semelparity may therefore not necessarily be only a correlated response to selection for early reproduction caused by high extrinsic adult mortality, as is frequently hypothesized; instead, it may also be a correlated response to natural selection at juvenile stages against traits that are correlated with adult reproduction. Selection on juvenile traits can be important determinants of the evolution of adult life histories.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1. Result of analyses of variance to compare morphology at the time the cutting predrought treatment was applied.

Table S2. Result of analyses of covariance to determine the contribution of morphological traits to the differential survivorship of high- and low-elevation populations under drought stress.