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1. Abstract.

Natural variation of reproductive lifespan has been reported in diverse biennial plant species. However, few studies in plants have investigated the adaptive significance of reproductive lifespan under natural conditions. Life history theory hypothesizes that monocarpy is an extreme case of reproductive cost, and the evolution of monocarpy is a byproduct of natural selection for maximal reproductive output. However, the modular viewpoint of plant structure, comparative studies, and previous genetic studies in animal species suggest a different hypothesis: reproductive lifespan might be influenced by reproductive effort as well as size at maturation, juvenile growth rates, and adult survival. In order to test whether and how natural selection favors monocarpy in biennial plant species, I conducted a demographic survey in 8 *Erysimum capitatum* natural populations including **a population in South Shanahan trail of Shanahan Ridge, City of Boulder.** This species shows natural variation in morphology and life histories along an altitudinal gradient.

2. Objectives and hypotheses.

Biennial plant species exhibit a monocarpic reproductive strategy. They reproduce during their second growing season, and die after the first reproductive episode (Bender et al., 2002). Polycarpic individuals, in contrast, survive after the first reproduction and reproduce again in a future growing season. Interestingly, natural variation in reproductive lifespan has been reported across populations in many biennial species (Falinska and Piroznikow, 1983;Verkaar and Schenkeveldt, 1984; Paige and Whitham, 1987; Bender et al., 2002; Bertin, 2001; Adams et al., 2004). Why is natural variation in reproductive lifespan so common?

Life history theory hypothesizes that reproductive lifespan is a result of adaptation to particular environmental conditions that influence age-specific survival and fecundity (Stearns, 1992). Lifetime fitness consists of diverse fitness components including adult survivorship, fecundity, juvenile survivorship, and age at maturation (Roff, 1992). Therefore, if trade-offs exist between adult survival rates and other fitness components, polycarpy does not always have higher lifetime fitness than monocarpy even though polycarpic individuals reproduce more often than monocarpic individuals. Monocarpy is, therefore, thought to be a byproduct of natural selection on traits which improve lifetime fitness other than prolonged reproductive lifespan (Stearns, 1992). Two key questions are, what kinds of traits are under direct selection, and how are these traits are correlated with reproductive lifespan.

Resource allocation models suggest that monocarpy is an extreme case of a reproductive cost, and that the major fitness advantage of monocarpy is an increased reproductive output during its single reproductive episode (Kelly and Davis. 1988). However, monocarpy of biennial plant species might have trade-offs with life history

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traits other than fecundity. In plant species, it is suggested that reproductive lifespan has negative correlations with juvenile growth rate and fecundity per reproductive episode, and a positive correlation with size (or age) at maturation (Bender et al, 2000; Franco and Silvertown, 1997). Moreover, adult survivorship was shown to have genetic correlations with juvenile survival (Partridge and Fowler 1992; Buck et al., 2000), juvenile growth rate (Dudycha and Tessier, 1999), size at first reproduction (Stearns et al., 2000), and early reproductive efforts (Rose, 1984) in animal species. This implies that increasing reproductive lifespan might have costs to juvenile fitness components.

For ecological situations in which reproductive lifespan varies across species or populations, one critical question is whether the variation is due to differences in the selective environment, differences in the genetic correlations among life history traits, or both. Natural populations of *Erysimum capitatum* in the Colorado Rocky Mountains provide an excellent opportunity to test for variable natural selection on reproductive lifespan. This species shows natural variation in reproductive lifespan: biennial monocarpic life cycle at low altitude and perennial polycarpic at high altitude environments. Biennial ecotypes show smaller size at maturation and higher reproductive effort per reproductive episode than perennial ecotypes (Price, 1987).

As a preliminary study, I conducted demographic survey of eight populations across National Forest area in Colorado. Following hypotheses were tested:

1. Do *E. capitatum* natural populations show an altitudinal gradient in morphological traits?
2. Do *E. capitatum* natural populations show an altitudinal gradient in life history traits?

3. Methods.

3.1. *Erysimum capitatum* as study species.

According to Rollins (1993), *Erytsimum spp.* is classified in Group V in Brassicaceae: siliques are 3 times longer than wide, and some branched trichomes range from once-forked to highly branched. They are characterized by appreseed, equal-armed, medifixed trichomes with two, three, or four (sometimes up to seven) branches, which are usually arranged with their long axes (for two-part hair) parallel to the long axis of the organ. Sepals are differentiated into well-marked blade and claw, with the blades spreading at right angles to the claws at anthesis.

E. capitatum (Brassicaceae) in the Rocky Mountains shows natural variation in morphological and reproductive lifespan along an altitudinal gradient. Taxonomic studies using Herbarium specimen suggested that it shows a monocarpic biennial habit at low elevations, but a polycarpic perennial habit in alpine environments (Price, 1987). Perennial individuals had been classified as a separate species (E. nivale or E. amoenum), but recent morphological and geographic studies suggested that they were ecotypes of E. capitatum (Price, 1987). In this paper, high and low altitude individuals are considered as ecotypes of *E. capitatum*, by following Price's classification.

Perennial ecotypes are characterized to have caudex, multiple stems from the base, and relatively short inflorescence. They generally have inflorescence less than 20 cm (Rossbach, 1958). Their leaves are not evenly distributed along the stem, but a few leaves arise from one spot of stem (Personal observation). In contrast, biennial races show tall inflorescence (-80cm) and they generally don't have caudex or stems.

3.2. Study populations and methods.

Along the altitudinal gradient, *Erysimum capitatum* natural populations were surveyed during the summer, 2004. A total of 8 populations were chosen at three different elevations in Colorado, USA (Table 1): three populations above the timberline (11,000ft), two populations at low elevation (6,000 – 7,500 ft), and three populations between timberline and low elevation. One of low elevation populations inhabit in South Shanahan trail of Shanahan Ridge, City of Boulder. In order to separate altitudinal variation from other geographic factors, three counties with three different altitudes were selected, and at least two populations with different altitudes were selected within each county.

Three to four demographic plots were constructed in each population, and six morphological traits were measured on individuals within each plot: leaf number (ln), rosette diameter (rd), heights of inflorescences (ht), number of inflorescences (in), flower number (fn), and silique number (sn) (Table 2). Three censuses were conducted from July to September in 2004. In order to compare morphological traits between different altitudes, it is important to use individuals at the same developmental stage. Adult morphological traits of high altitude populations were measured after most flowers died.

Table 1. Natural populations in Colorado.

Population	Location	Altitude
B1	Univ. of Colorado Mountain Research Station Alpine Lab, Boulder Co.	11,514 ft.
B2	Meadow behind Univ. of Colorado Mountain Research Station, Boulder Co.	9,742 ft.
B3	**South Shanahan trail of Shanahan Ridge, City of Boulder.**	**6,111 ft.**
C1	Loveland pass, Clear Creek Co.	11,900 ft
C2	Evans Mt.(picnic ground-ponder point), Clear Creek Co.	10,280 ft.
C3	Lookout Mt., Clear Creek Co.	7,341 ft.
G1	Emerald lake, Gunnison Co.	10,707 ft
G2	Snodgrass trail at the head of Gothic Rd, Gunnison Co.	9,570 ft

Table 2. Measured traits.

Abbrev.	Trait.
ln	leaf number
rd	rosette diameter
ht	height of inflorescence
in	number of inflorescences
fn	flower number
sn	silique number

4. Results.

1.a. Morphological and life history traits.

I verified that populations differed across altitude in key life history traits associated with reproductive lifespan. Reproducing individuals at high altitude had more than one vegetative rosette without an inflorescence (fig 1 and fig 2). After the shoot apical meristem of each rosette was used to produce an inflorescence, the rosette leaves connected to the inflorescence dried out. However, leaves of non-reproductive rosettes remained alive with green color (fig1. (a)). In contrast, low altitude ecotypes generally did not make additional rosettes. Instead, they produced one big inflorescence (fig 1. (b)), and their leaves were dried out.

Survivorship of reproducing individuals was decided whether rosette leaves were maintained at the time of census in September, 2004. At high elevation, over 92% of reproducing individuals survived past reproduction. In contrast, only 8% of the reproducing adults maintained vegetative structures at low elevation. Over 90% of non-reproductive plants survived at both low and high elevation.

Compared to the high altitude ecotypes, the low altitude ecotypes produced significantly fewer and longer inflorescences (fig 2). They produced larger number of siliques per inflorescence than high altitude ecotypes. However, total silique number was not significantly different because high altitude ecotypes had more inflorescence than low altitude ecotypes. Therefore, it does not appear that monocarpic individuals have higher fecundity per reproductive episode than polycarpic individuals when each is grown in its native environment.

Non-reproducing plants at high altitudes had a significantly larger number of leaves with smaller diameter than those at low altitudes (fig 4). If this cline is maintained until reproduction, low altitude ecotypes might start to reproduce with smaller number of rosette leaves. In addition, it was suggested that slow-growing species tend to have leaves with small size compared to rapid-growing relatives (Ardent, 1997). If *E. capitatum* populations follow the same pattern, juvenile growth rates of high altitude ecotype will be lower than that of low altitude ecotype.

1.b. Mechanism to induce different reproductive lifespan.

In high altitude ecotypes of *E. capitatum*, vegetative rosettes were maintained during reproductive periods (fig 1). In contrast, low altitude ecotypes did not produce any vegetative rosettes during or before reproduction. The primary shoot apical meristems of reproducing rosettes were used to produce inflorescence. According to the meristem limitation hypothesis, monocarpic plants use all available meristems for reproduction, but polycarpic plants maintain vegetative apical meristems during reproduction. This implies that vegetative rosettes are important to maintain vegetative apical meristems for survival in *E. capitatum*.

The detailed structure of vegetative rosettes was investigated using dissection microscope. In perennial ecotypes, new vegetative rosettes are produced from the primary rosette or a stem (fig 3). At both organs, there is always a remnant of dead leaf just below a vegetative rosette, which suggests that a vegetative rosette is produced at the axil of a leaf (fig3. (c)). In addition, stems are also produced at the axils of primary rosette leaves (fig3. (g)). These observations suggest that a key trait of perennial ecotypes is to produce and maintain vegetative axillary rosettes during reproductive event.

5. Conclusions and future plan.

Demographic survey in 2004 suggested that *Erysimum capitatum* populations show altitudinal gradients in natural environments. At low elevations, it shows a monocarpic biennial habit, but in alpine environments, it exhibits a polycarpic perennial habit (Price, 1987). In addition, the number of leaves and inflorescences of low altitude ecotypes were smaller, but their sizes were larger than high altitude ecotypes. At low altitude, silique number per inflorescence was large, but total silique number was not significantly different from high altitude ecotypes.

Differentiation of two distinct populations provides a working hypothesis that reproductive lifespan of *E. capitatum* adapts to local environments. By following life histories of each population, it can be tested whether natural selection acts on reproductive lifespan. The transition matrix and projected growth rate of each population can be constructed based on demographic survey (McGraw and Caswell, 1996; Waser and Price, 1985). By comparing the growth rate of each population, theoretical predictions on the evolution of reproductive lifespan can be tested. Moreover, transition matrices also can provide another useful parameter, elasticity of each matrix element. By analyzing elasticity, it is possible to detect the relative contributions of survivorship, growth and fecundity of each life stage to the population growth (Dinnetz and Nilsson, 2002). The elasticities of matrix components in polycarpic populations can be evaluated at high and low altitude environments. If natural selection acts on reproductive lifespan, adult survivorship will contribute to population growth more than juvenile survivorship or fecundity of first reproduction at high altitude environment. In contrast, elasticity of adult survivorship will be lower than that of other components at low altitude environments. Therefore, in order to test natural selection on reproductive lifespan, continued demographic studies are required.

One of the hidden assumptions in life history theory is that variation in life history traits has a genetic basis (Stearns, 1992). Without a genetic basis, population differentiation is a simple result of environmental differences. Therefore, assessment of genetic basis is an essential step to test adaptive hypotheses (Reznick and Travis, 1996). A reciprocal transplant experiment in natural habitat of *E. capitatum* is needed to test genetic basis of variation in reproductive lifespan.

Fig 1. *Erysimum capitatum* in reproductive stage. (a) an individual from the Gothic high altitude population. Leaves of the reproducing rosette start to die (reddish yellow), but leaves of non-reproducing rosette remain green. (b) an individual from Clear Creak low altitude population. All leaves are dried out.

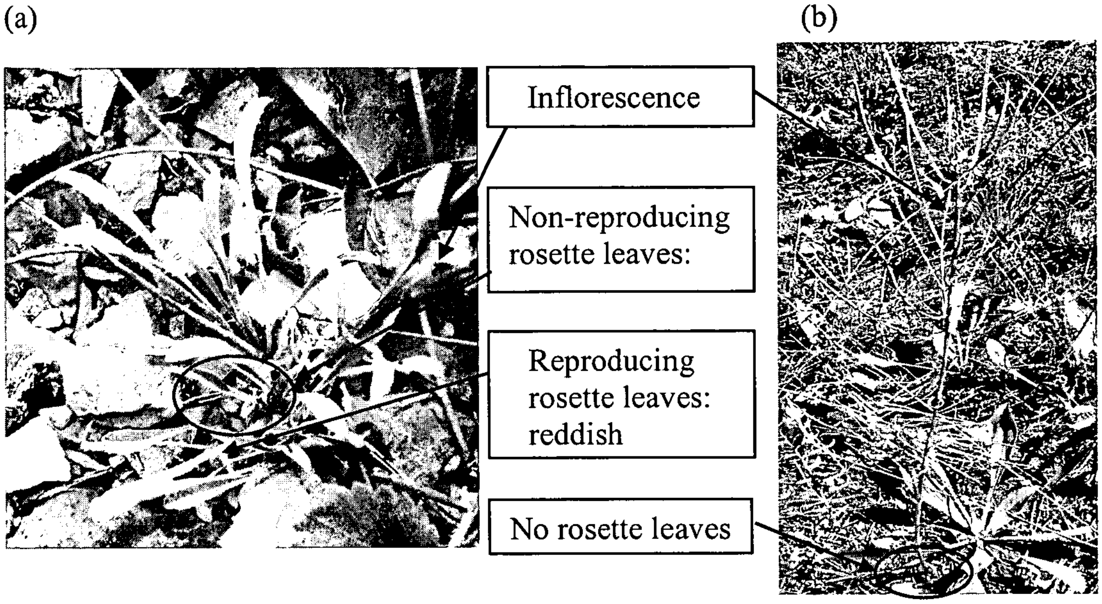
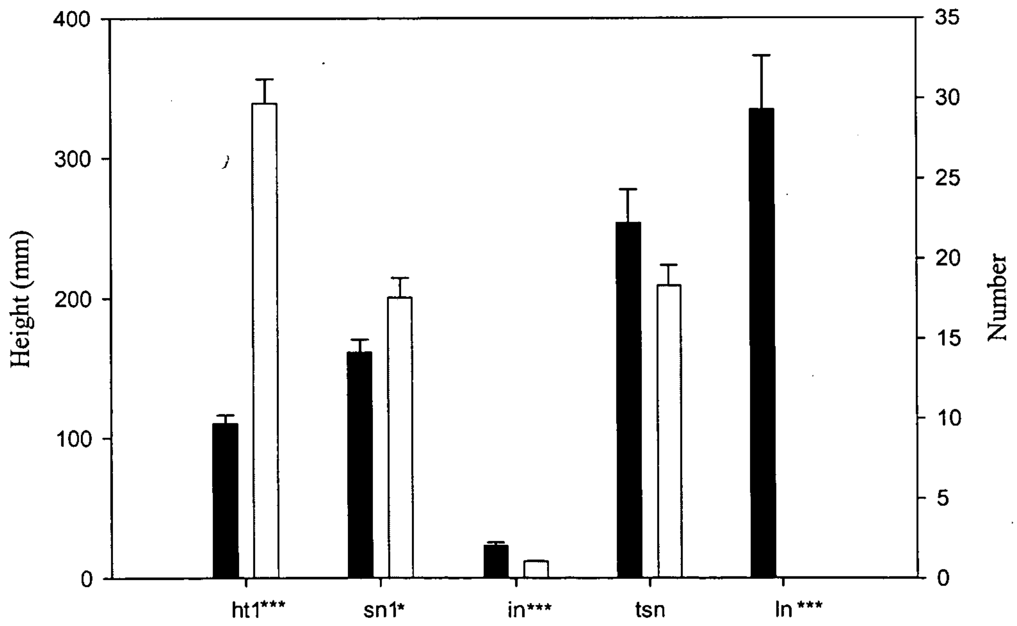


Fig 2. Morphological differences between high and low altitude populations



■ High altitude population, ☐ Low altitude population.
ht1: height of the highest inflorescence, trep1: silique number of the highest inflorescence, in: the number of inflorescence, tsn: total number of siliques, ln: number of rosette leaves. Statistical significance were shown as the number of asterisks (***: 0.001, **: 0.01, *: 0.05)

Fig 3. Production of vegetative axillary rosettes and stems in high altitude ecotypes. (a) –(c): photograph of axillary rosettes from the axils of primary rosette leaves. (d)-(g): production of stems from the axils of primary rosette leaves.

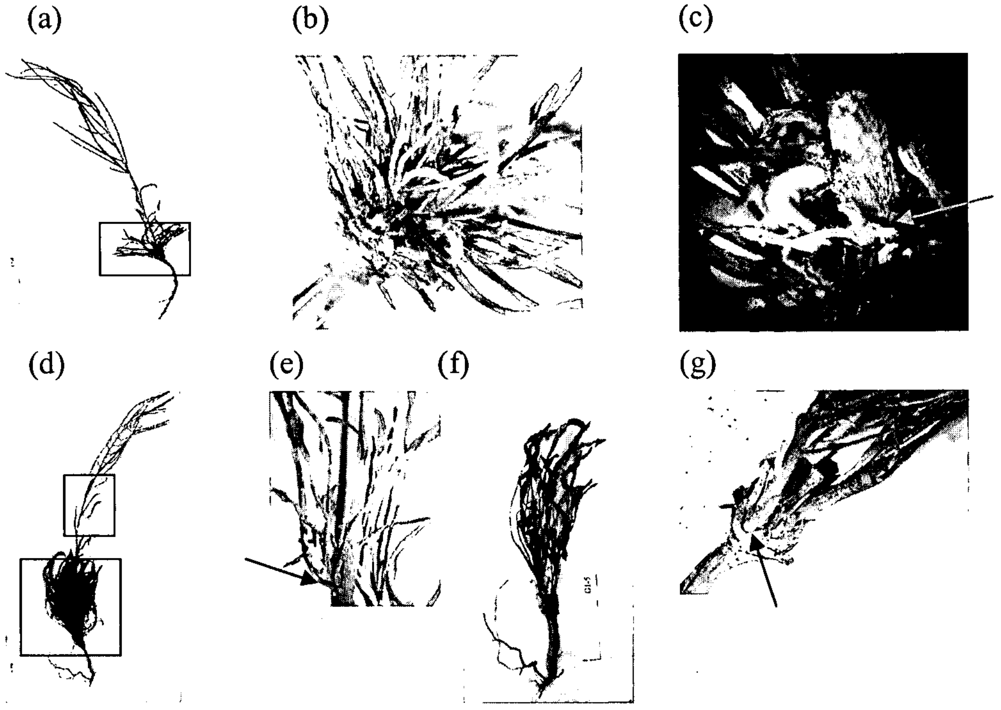
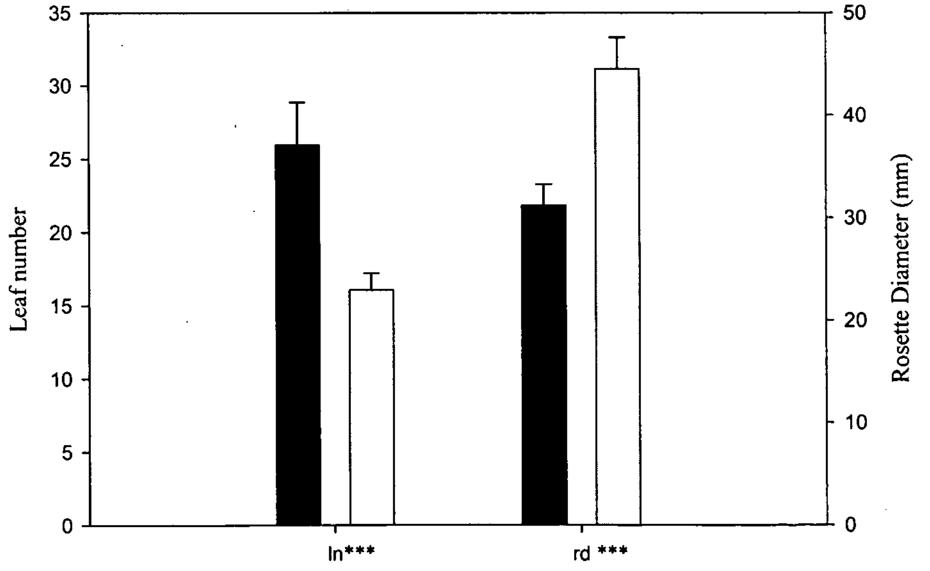


Fig 4. Altitudinal variation in vegetative traits.



■ High altitude population, □ Low altitude population.
ln: number of rosette leaves, rd: maximum rosette diameter.

Statistical significance were shown as the number of asterisks (***: 0.001, **: 0.01, *: 0.05)

Reference cited.

Adams, C. A., J. Baskin, and C. Baskin. 2003. Comparison of the morphology, flowering phenology, and life cycle type in plants of *Grindelia lanceolata* (Asteraceae) from cedar glades in middle Tennessee and northern Alabama: A common garden study. Sida Contributions to Botany 20:1059-1071.

Bender, M. H., J. M. Baskin, and C. C. Baskin. 2002. Phenology and common garden and reciprocal transplant studies of *Polymnia Canadensis* (Asteraceae), a monocarpic species of the North American Deciduous Forest. Plant Ecology 161: 15-39.

Bender, M. H., J. M. Baskin, and C. C. Baskin. 2000. Age of maturity and life span in herbaceous, polycarpic perennials. Botanical Review 66:311-349.

Bertin, R. I. 2001. Life cycle, demography, and reproductive biology of herb Robert (*Geranium robertianum*). Rhodora 103:96-116.

Buck, S., J. Vettraino, A. G. Force, and R. Arking. 2000. Extended longevity in *Drosophila* is consistently associated with a decrease in larval viability. Journal of Gerontology A 55:B292-B301.

Dinnetz, P. and T. Nilsson. 2002. Population viability analysis of *Saxifraga cotyledon*, a perennial plant with semelparous rosettes. Plant Ecology 159:61-71.

Dudycha, J. L. and A. J. Tessier. 1999. Natural genetic variation of lifespan, reproduction, and juvenile growth in *Daphnia*. Evolution 53: 1744-1756.
Stearns, S. C., M. Ackermann, M. Doebeli, and M. Kaiser. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. PNAS 97:3309-3313.

Falinska, K., and E. Piroznikow. 1983. Ecological structure of *Geranium robertianum* L. Populations under natural conditions and in the garden. Ekologia Polska 31: 93-121.

Franco, M., and J. Silvertown. 1997. Life history variation in plants: an exploration of the fast-slow continuum hypothesis. In Plant life histories. Ed. by J. Silvertown, M. Franco, and J. L. Harper. Cambridge Univ. Press. Cambridge, UK.

Kelly, M. O. and P. J. Davis. 1988. The control of whole plant senescence. CRC Critical Reviews in Plant Science 7: 139-173.

McGraw, J. B., and H. Caswell, 1996. Estimation of individual fitness from life-history data. American Naturalist 147: 47-64.

Paige, K. N. and T. G. Whitham. 1987. Flexible life history traits: shifts by scarlet gilia in response to pollinator abundance. Ecology 68: 1691-1695.

Partridge, L. and K. Fowler. 1992. Direct and correlated response to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46: 76-91.

Price, R. A. 1987. Systematics of the *Erysimum capitatum* alliance (Brassicaceae) in North America. Ph.D. thesis. University of California, Berkeley.

Reznick, D. and J. Travis. 1996. The empirical study of adaptation in natural populations. in Adaptation ed. by M. R. Rose and G. V. Lauder. Academic Press. California, USA.

Roff, D. A. 1992. The evolution of life histories. Chapman & Hall. New York, USA.
Rollins, R. C. 1993. The cruciferae of continental north America. Stanford University Press. California, USA.

Rossbach, G. B. 1958. The genus Erysimum in North America north of Mexico – a key to the species and varieties. Madrono 14: 261-267.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosohpila melanogaster*. Evolution 38:1004-1010.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press. Oxford, UK.

Verkaar, H. J. and A. J. Schenkeveld. 1984. On the ecology of short-lived forbs in chalk grasslands: semelparity and seed output of some species in relation to various levels of nutrient supply. New Phytologist 98:673-682.

Waser, N. M., and M. V. Price, 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae): evidence for local adaptation. American Journal of Botany 72: 1726-1732.