CRYPTIC SEED HETEROMORPHISM IN *PACKERA TOMENTOSA* (ASTERACEAE): DIFFERENCES IN SEED MASS CHARACTERISTICS AND GERMINATION. Lindsay D. Leverett. Director of thesis: Claudia L. Jolls, Ph.D. Department of Biology. March, 2012.

Germination requirements of seeds can dictate when and where plant offspring establish. Microsites available for germination vary spatially and temporally in factors such as temperature and moisture; thus, the production of seeds with identical requirements may limit germination. When seed mass influences germination and offspring establishment, the production of seeds with a range of sizes encourages differential behavior in progeny. Seed heteromorphism, the production of two or more seed types with different forms and/or behaviors by the same plant, may be "cryptic" when seed types have different behaviors but similar morphologies. Although rarely documented, cryptic seed heteromorphism may be widespread among plant taxa. The production by a plant of seeds with variable mass or heteromorphism may increase the number of microsites favorable for germination.

I investigated seed mass variation and seed heteromorphism in *Packera tomentosa* (Michx.) C. Jeffrey (woolly ragwort, Asteraceae), a clonal plant found in disturbed habitats in the coastal plain of the southeastern U.S. Like most members of the Asteraceae, *P. tomentosa* displays flowering heads that contain disc and ray florets, which produce central and peripheral seeds, respectively. Seeds were collected from 50 purported clones of *P. tomentosa* at East Carolina University's West Research Campus (WRC), Pitt County, North Carolina. Seed mass was compared among- and within-genetic individuals as well as between floret types of a flowering head. Amplified fragment length polymorphism (AFLP) profiles confirmed that all 50 clones were unique genetic individuals or "genets", verifying that seedling recruitment does occur in this population of *P. tomentosa*. I compared total mass and allocation to the embryo and pericarp between central and peripheral seeds. An initial study investigated germinability and speed of germination for both seed types in controlled conditions. I then tested the germination

response of central and peripheral seeds to frequent, intermediate, and infrequent watering intervals. A final study determined whether germination speed or success of central and peripheral seeds was influenced by aging and/or cold stratification.

Overall, seed mass of *Packera tomentosa* was highly variable among- and within-plants. Larger seeds exhibited faster, higher germination. Central and peripheral seeds did not have different morphologies, but differed in mass characteristics and germination. Central seeds were 11% heavier with 80% larger embryos than were peripheral seeds, whereas peripheral seeds allocated 17% more of total mass to the fruit coat than did central seeds. Differences in total mass between seed types appear to be driven by embryo size. Central seeds germinated at a higher proportion than did peripheral seeds (74.5 vs. 36.0%, respectively) and germinated faster than peripheral seeds (32.8 vs. 36.2 d, respectively). Differences in germination may be due to pericarp thickness or embryo size. Central seeds exhibited greater germination success in frequent and infrequent watering intervals, but not in the intermediate watering interval. Both seeds types showed lowest germination in response to infrequent watering, suggesting germination success decreases in drought conditions. Germination speed increased in central seeds following aging; thus, central seeds after-ripen. In contrast, peripheral seeds germinated faster after cold stratification, suggesting they delay germination and are stimulated by cooler temperatures.

Cryptic seed heteromorphism occurs in *Packera tomentosa,* supporting the suggestion that this trait may be more common than is documented, particularly in the Asteraceae. In *P. tomentosa*, variation in germination behavior resulting from seed mass variation and seed heteromorphism may spread the risks associated with germination among many offspring phenotypes, potentially functioning as bet-hedging strategies and providing success in the unpredictable environments this species inhabits.

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Lindsay D. Leverett

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Dedicated to Michael Woods, Ph.D.,
for introducing me to the exciting field of botany

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INTRODUCTION

Seeds, the sexual offspring of plants, represent important means of spatial and temporal dispersal. Their germination requirements dictate when and where plant offspring can establish. In unpredictable or heterogeneous environments, microsites available for germination vary both spatially and temporally in conditions such as temperature, soil microtopography, and light intensity, all of which can affect seed and seedling success (Sheldon, 1974). Additionally, seeds in the field are exposed to fluctuating cycles of hydration and dehydration (Harper, 1977; Kagaya *et al.*, 2005). Soil moisture variability, often resulting from sporadic precipitation, influences seed germination and is receiving more attention given projected rises in drought intensity and duration as a result of global climate change (Cornaglia *et al.*, 2005; Fay and Schultz, 2009). Variability in soil features can limit plant reproductive success if all offspring require identical conditions for germination. Variation in seed size, which impacts almost all features of germination and seedling establishment (Harper *et al.*, 1970), can ensure some offspring encounter favorable conditions. Most importantly, seed size has consequences for the timing of germination, which in turn influences fecundity and survival (Simons and Johnston, 2006 and references therein). The production of seeds with a range of phenotypes can reduce temporal variation in fitness, thus limiting the likelihood of zero survival in a given season (Childs *et al.*, 2010). When variable seed size impacts germination and becomes adaptive, the strategy can function as diversified bet hedging, in which risk is spread among many offspring phenotypes (Simons, 2011).

High seed mass variation has been documented in many species, with coefficient of variation (CV) values for seed mass commonly > 5%, considered "large" for a biological trait (Thompson, 1984). For example, the average CV for seed mass is 33% in *Convallaria majalis*

(Liliaceae, Eriksson, 1999) and 26% in *Lomatium grayi* (Umbelliferae, Thompson, 1984). This variation appears to be common across taxa with no consistent ecological correlates (Michaels *et al.*, 1988). Within a population, substantial variation in seed mass may be found among plants and within plants (Thompson, 1984), as well as within individual fruits (Stanton, 1984). Seed mass variation within an individual may be independent of genetic differences among seeds (Tweney and Mogie, 1999).

"Multiple strategies", which allow an individual plant to concurrently control two or more structures that perform the same function (Lloyd, 1984), also can promote persistence in unpredictable environments. Seed heteromorphism or heterocarpy, the production by a single plant of two or more types of diaspores with different forms and/or behaviors (Venable, 1985a), is one such strategy. Differences among seed or fruit morphs arise due to somatic responses during their development and usually include contrasting mass, dispersal, and germination patterns (Sorensen, 1978; Imbert, 2002). Seed morphs of heteromorphic taxa often germinate differently in response to environmental factors, such as temperature (*Senecio jacobaea*, Asteraceae, Baker-Kratz and Maguire, 1984), light (*Suaeda acuminata*, Chenopodiaceae, Wang *et al*., 2012), and salinity (*Chenopodium album*, Chenopodiaceae, Yao *et al.*, 2010). These dissimilar requirements of seed morphs increase the chance that some offspring will encounter favorable microsites. Seed heteromorphism is associated with occurrence in unpredictable habitats, with most cases of seed heteromorphism occurring in annuals (Mandák, 1997; Cruz-Mazo *et al*., 2009). Although studied primarily in annuals, particularly of deserts, seed heteromorphism has been documented in a few perennial species (e.g. *Prionopsis ciliata*, Asteraceae, Gibson, 2001; *Leontodon autumnalis*, Asteraceae, Picó and Koubek, 2003).

Seed heteromorphism is "cryptic" when seed types display different ecological behaviors unaccompanied by obvious morphological dissimilarities. Cryptic seed heteromorphism may be more common in angiosperms than documented. By definition, cryptic heteromorphism may be overlooked due to a lack of morphological distinction among seeds and the difficulty in detecting contrasting behaviors (Silvertown, 1984; Venable, 1985a). In documented cases of cryptic heteromorphism, these behaviors include contrasting dispersal (*Sparganium emersum*, Sparganiaceae, Pollux *et al*., 2009) and different growth in plants derived from seed types (*Ambrosia artemisiifolia*, Asteraceae, Fumanal *et al.*, 2007).

Although documented in many plant families, seed heteromorphism is most common in the Asteraceae, which contains roughly 63% of species known to exhibit seed heteromorphism (Imbert, 2002). The Asteraceae produce dry, single-seeded fruits called achenes, consisting of a pericarp (fruit coat) and an embryo. Each fruit is a "cypsela", an achene derived from an inferior ovary (Marzinek *et al.*, 2008). Since the Asteraceae produce fruits that function much like seeds, seed heteromorphism in the family is referred to as heterocarpy (i.e. different fruits). The prevalence of heterocarpy in the Asteraceae may be due to the anatomy of the composite head, which is typically composed of an outer whorl of ray florets and inner whorls of disc florets (Zohary, 1950; Venable, 1985a). Morphological differences among the fruits can include color, shape, and trichome characteristics, but most relate to size and pappus structures (Zohary, 1950; Imbert, 2002). Generally, two different morphs are produced: central cypselae derived from interior disc florets and peripheral cypselae derived from outer ray florets. The central morphs commonly are smaller and more dispersive than peripheral morphs (Imbert, 2002). The larger size in peripheral morphs has been attributed to the centripetal development of the head, which results in the production of ray florets and their peripheral fruits first (e.g. McEvoy 1984).

Central seeds are heavier than peripheral seeds, however, in some heterocarpic Asteraceae (e.g. *Picris radicata,* Ellner and Shmida, 1984; *Bidens pilosa*, Rocha, 1996). Size differences between seed morphs generally relate to embryo size (Imbert, 2002).

In heterocarpic Asteraceae, the most noted divergence in behavior between morphs occurs in germination. The conditions required for germination usually are more restricted for one morph than the other (Venable, 1985a). Generally, central morphs germinate quickly while peripheral morphs show dormancy or delayed germination (Imbert, 2002). Dormancy provides a buffer against unfavorable conditions (Venable and Lawlor, 1980); thus, the production of dormant peripheral morphs allows heterocarpic plants to disperse progeny in time. While continuous seed mass variation creates a range of offspring behavior, seed heteromorphism involves discrete classes of seeds that may have opposing roles such as population maintenance by non-dispersive, dormant seeds vs. colonization by dispersive, highly germinable seeds. Both strategies broaden the range of conditions under which plant offspring can achieve successful germination, however, promoting the ability to reproduce in stressful or unpredictable habitats (Fenner and Thompson, 2005).

I investigated aspects of seed ecology of *Packera tomentosa* (Michx.) C. Jeffrey (Asteraceae) (Jeffrey, 1992), woolly ragwort, a perennial species in tribe Senecioneae, subtribe Senecioninae (Bain and Golden, 2000; Pelser *et al*., 2007; Fig. 1). The genus *Packera* Á. Löve and D. Löve likely originated in Mexico (Bain and Golden, 2000), evolving from the ancestral lineage of *Senecio* (Knox and Palmer, 1995). The genus belongs to the monophyletic Aureoid group of *Senecio* characterized by pollen with a perforated exine ("helianthoid pollen", Bain and Walker, 1995). Species of *Packera* are readily interfertile, obligately outcrossing, and efficient at seed dispersal (Barkley, 1988). The genus is closely related to the genera *Pericallis* and

Fig. 1. *Packera tomentosa* flowering heads showing disc florets, which produce central seeds (C), and ray florets, which produce peripheral seeds (P). Photo credit: James P. Tumulty.

Emilia (Bain and Golden, 2000; Pelser *et al.*, 2007). *Packera tomentosa* (= *Senecio tomentosus* Michx.; *S. alabamensis* Britton ex Small; *Cineraria integrifolia* Jacquin ex Willdenow var. *minor* Pursh) is a clonal species native to North America distributed primarily within the coastal plain of the southeastern United States, where it inhabits open meadows, roadsides, and sandy, shallow soils (Radford *et al.*, 1968; eFloras, 2011). Plants (30-60+ cm tall) occur as stands of clustered rosettes of lanceolate-elliptic leaves, 40-120+ mm long by 20-50+ mm wide, with crenate to serrate-dentate margins, often with a floccose tomentum proximally. *P. tomentosa* reproduces vegetatively, producing offshoots of rosettes (ramets) connected by belowground rhizomes or occasionally aboveground stolons. Woolly ragwort also reproduces sexually as a flowering stalk of 10-30+ heads in a corymbiform array. Each yellow-gold head is comprised of 5-14 outer ray florets and 50-60+ inner disc florets. *P. tomentosa* flowers from (Mar-) May-early June (Deborah Trock, eFloras, 2011). The cypselae of *P. tomentosa* are hispid and bear a calyx modified as a pappus of capillary bristles 5-7 mm long (Fig. 2). The pappus and pericarp of Asteraceae fruits can collectively contribute to dispersal and germination of the fertilized ovule, allowing the whole fruit to function as a seed; hereafter, the cypsela is referred to as a seed.

Packera tomentosa is associated with disturbed and unpredictable habitats (eFloras, 2011), and can aggregate in depressions and ditches where water may be more abundant. Its close relative, *Senecio jacobaea* (Asteraceae), is a well-studied heterocarpic species (e.g. McEvoy, 1984). Germination studies of heterocarpic species often test for different responses of seeds to light intensity, temperature, and/or salinity (see above), but fewer attempt to measure response to moisture availability (but see Venable, 1985b). I hypothesized that seed mass variation and/or seed heteromorphism in *P. tomentosa* may broaden the range of favorable

Fig. 2. Central (top row) and peripheral (bottom row) seeds of *Packera tomentosa* with intact pappus (scale bar = 1 mm). Photo credit: Corey Doughty.

microsites for germination, defined in part by water availability, and promote persistence in the highly disturbed habitats where *P. tomentosa* is successful. I used seed mass characteristics and germination behavior studies to test for seed mass variation and heterocarpy in *P. tomentosa*. Specifically, I asked:

(1) How variable is seed mass and do central and peripheral seeds differ in mass?

(2) Does seed mass or position influence germination?

(3) Do central and peripheral seeds germinate differently in varying watering intervals?

(4) Does aging or vernalization influence germination in central and/or peripheral seeds?

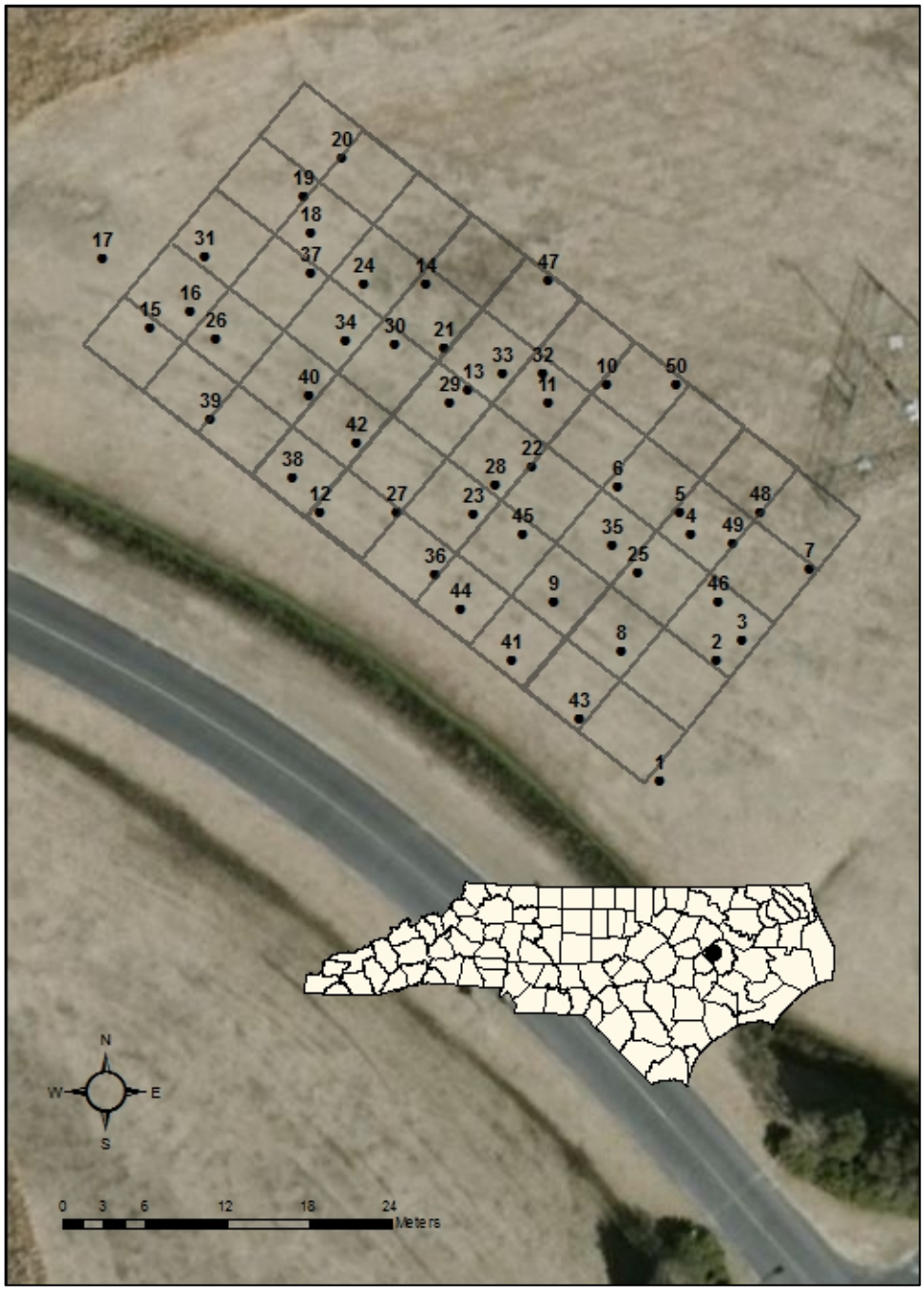
MATERIALS & METHODS

Study site, seed collection, and storage

I studied a population of *Packera tomentosa* located at West Research Campus (WRC), an ecological research and education facility owned by East Carolina University. Formerly a Voice of America (VOA) site, the 235 ha tract of land is located northwest of Greenville, western-central Pitt County, North Carolina, USA (Chester, 2004). Historically, WRC most likely was a longleaf pine mineral flat with sections of mixed-pine upland. Mowing was implemented between the early 1960s and 1990s to control woody vegetation and access roads were added with ditches to drain water (Chester, 2004). Approximately 60% of WRC is jurisdictional wetland and soil is generally nutrient-poor (Goodwillie and Franch, 2006). *P. tomentosa* is dominant in the locations disturbed by regular mowing, often forming nearly monospecific stands. In areas without regular mowing *P. tomentosa* generally is absent.

A 50 m × 25 m plot was established in a cleared area near an access road to monitor flowering and seed maturation (35°38'1.522"N, 77°29'6.792"W; Fig. 3). The density of *Packera tomentosa* rosettes was approximately 50 per m^2; roughly 30% were flowering. Heads of *P. tomentosa* were collected in May 2011 as they matured, using structured sampling to investigate seed mass characteristics at multiple levels within the population: among clonal individuals, among rosettes (ramets) of the same clonal individual, and among heads of the same flowering stem. I selected distinct groups of plants that I presumed to be clones (genetic individuals) separated by at least 1 m when possible. I gently excavated the rhizome to determine which rosettes were part of the same clonal individual (genet). Genetic identities later were confirmed using amplified fragment length polymorphism (AFLP, see below) profiles generated from leaf tissue collected from each of the rosettes. I collected three heads per

Fig. 3. Map of 25 × 50 m sampling plot with 5 m^2 subplots located at West Research Campus (WRC), located in western-central Pitt County, North Carolina (see NC state inset). Numbered points represent *Packera tomentosa* clones used in this study. Map created by Jason C. Paxton, using ArcGIS 10 software (ESRI, Redlands, CA) and GPS coordinates of clones and plot corners.



flowering stem, three flowering stems per clone for 37 clones and one flowering stem per clone for 13 clones. Heads were stored intact at room temperature prior to dissection, determination of seed weight, and germination experiments.

Confirmation of genetic identities

I used amplified fragment length polymorphisms (AFLP) profiles to confirm that the 50 clones sampled were genetically distinct. Additionally, I used samples from the leaves of 22 clones to confirm that rosettes belonged to the same clone. AFLP is a rapid, PCR-based tool used for genotyping or fingerprinting individuals (Vos *et al*., 1995; Meudt and Clarke, 2007). Leaf tissue samples were washed and dried prior to storage at –80 °C. DNA extraction and isolation from leaf tissue were performed using a CTAB/chloroform protocol modified from that of Doyle and Doyle (1987). Each tissue sample was digested and ligated to adaptors in an 11-μL reaction containing 5.5 μL of diluted DNA samples, 1 μL T4 ligase buffer, 1 μL 0.5 M NaCl, 0.5 μL BSA, 1 μL MseI adaptor, 1 μL EcoRI adaptor, and 1 μL of enzyme mix (0.1 μL T4 ligase buffer, 0.1 μL 0.5 M NaCl, 0.1 μL BSA, 0.4 μL dH_2O, 0.3 μL EcoRI, 0.1 μL MseI, and 0.1 μL T4 ligase enzyme). Reactions were incubated at 37 °C for 6 h then diluted in 90 μL of TE buffer.

Preamplification reactions were carried out as 16-μL reactions containing 2 μL of the diluted digestion-ligation reaction, 5.4 μL dH_2O, 8 μL 2X PCR buffer, and 0.3 μL each of MseI and EcoRI preamplification primers containing a single selective nucleotide. Preamplification cycles were: 2 min at 72 °C; 30 cycles of 30 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C; and 10 min at 60 °C. Following preamplification, reactions were diluted in 67 μL $TE_{.01}$ buffer. Final selective amplifications were performed as 12.5-μL reactions consisting 2.5 μL of the

preamplification reaction, 3.5 μL dH_2O, 6.3 μL 2X PCR buffer, and 0.3 μL each of MseI-CTG and EcoRI-ACC selective primers. Final selective amplification cycles were as follows: 2 min at 94 °C; 13 cycles of 30 s at 94 °C, 30 s at 65 °C (with a reduction by 0.7 °C per cycle), and 2 min at 72 °C; 24 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 56 °C; and 10 min at 72 °C.

The AFLP profile for each sample was analyzed and scored using GeneMapper v4.1 (Applied Biosystems, Foster City, CA), with peaks (fragments) scored as either present or absent. The analysis yielded 88 polymorphic loci; I excluded ambiguous loci, narrowing the list to 33 easily resolvable loci. Presence/absence data were used to construct a genetic distance matrix using GenAlEx 6.41 (Peakall and Smouse, 2006). I then examined these data to confirm that presumed ramets of the same clone of *Packera tomentosa* indeed had identical genotypes (i.e. identical at all 33 loci) and that each presumed clone had a unique genotypic fingerprint (i.e. not identical at all 33 loci).

Seed mass characteristics

Five mature, filled seeds per position from each head were chosen randomly from 15 clones (three heads per flowering stem, three flowering stems per clone) in June 2011. Seeds were gently squeezed with forceps to ensure they were filled. I removed the pappus and weighed each seed individually to the nearest 0.0001 mg (Cahn model E-15, Cerritos, CA, USA). To determine whether central and peripheral seeds differed in mass allocation, I dissected seeds into pericarp and embryo. Pericarps were massed individually to the nearest 0.0001 mg. I inferred embryo mass from these measurements [(total mass) – (pericarp mass)] and determined the proportion of total mass allocated to each structure [(pericarp mass) / (total mass); (embryo mass) / (total mass)].

Germination study #1: Central vs. peripheral seeds

An initial study was conducted to gather basic germination data for central and peripheral seeds of *Packera tomentosa*, asking whether position and/or maternal clone (genet) influence germination behavior. Filled seeds were used for all germination studies. The seeds had been stored dry at room temperature for approximately 1-1.5 mo when germination trials began. Seeds of the same position (central or peripheral) and same maternal clone were placed in Petri dishes on autoclaved sand in groups of five (40 and 39 dishes of central and peripheral seeds, respectively). I grouped seeds by maternal clone to control for potential differences among clones. Little is known about the germination requirements of *P. tomentosa*, but one study suggested that seeds require at least 12 h of light to germinate and achieve greatest germination following a short aging period (Chapman and Jones, 1971). Seeds were exposed to 16 h light (25 °C) and 8 h dark (15 °C) per d at 40-45% relative humidity for 70 d (Percival Scientific, Model AR-41L3, Perry, IA, USA). Seeds were watered and checked daily for emergence of the radicle through the pericarp, my criterion for germination.

Germination study #2: Influence of position, clone, mass, and watering interval

I conducted a second germination study using massed seeds to determine whether position, maternal clone (genet) and/or mass affect germination in *Packera tomentosa*. I also wanted to determine whether central and peripheral seeds differ in germination behavior, i.e. whether they respond differently to frequent, intermediate, and infrequent watering intervals, in germinability and the rate of germination. For this study, seeds had been stored dry at room temperature for approximately 1-1.5 mo. I used central and peripheral seeds from 12 clones. The germination protocol was identical to the study above, but with four seeds per dish and a

study duration of 78 d. Each dish contained four seeds of the same position from the same clone (N = 96 seeds, 48 each central and peripheral). This design was repeated across three different watering intervals based on the number of days between watering events: 0 d (***0-d***, frequent watering), 1 d (***1-d***, intermediate watering), and 3 d (***3-d***, infrequent watering). Sand remained saturated in the ***0-d*** interval and relatively moist in the ***1-d*** interval. The substrate in the ***3-d*** interval dried between watering events. Seeds were watered according to the treatment interval and checked daily for emergence of the radicle.

Germination study #3: Influence of seed aging and vernalization

Beginning late September 2011, *Packera tomentosa* seeds were moist-stratified at 2 °C for 30 d ("vernalized" seeds hereafter) to determine whether seeds exhibit dormancy at time of dispersal. It has been suggested that only a short cold period is necessary to break dormancy for this species (Chapman and Jones, 1971). I used central and peripheral seeds from six clones, with four seeds of the same position and clone per dish (N = 96 seeds, 48 each central and peripheral). To ensure any effects on germination behavior in this study were due to vernalization and not simply aging, I assembled another matched complement of 96 seeds (48 each central and peripheral) that had been stored dry at room temperature for the same amount of time ("aged" seeds hereafter). The aged seeds also were used to test for the effects of aging on germinability by comparing them to the seeds used in the ***0-d*** interval from the previous study. Seeds from the ***0-d*** study used for comparison in this study are hereafter referred to as "control" seeds. Aged and vernalized seeds were used from the same six clones and germinated simultaneously. Temperature, photoperiod, and relative humidity for both these studies were the

same as detailed above. Seeds were watered and checked for emergence of the radicle daily for 78 d.

Data analysis

I analyzed seed mass variation using a mixed-model nested ANOVA, treating "Position" as a fixed effect and all other levels as random effects. Partial *eta* squares were used to compare the relative amount of variance explained by each level in the population. In the nested model, all levels were tested against the mean squares error term (MS_{error}) except "Genet", which was tested against the mean squares of its subsequent level. Biomass allocation data were compared between seed types using Welch's t-tests. For the first germination study, I compared the number of days required for germination and the proportion of seeds that germinated using ANOVA, treating "Position" as a fixed effect and "Genet" as a random effect. Each Petri dish served as one replicate. For the second germination study, I calculated the germination velocity for each group (each of the two seed positions in each of the three watering intervals) to generate a standardized number for comparing the speed of germination among groups. Germination velocity is calculated as $\sum G/t$, where G is the cumulative percent germination at two-day intervals and t is the total number of days sampled (Khan and Ungar, 1997); thus, the index measures germination over time. Higher index values represent more rapid germination, with the highest possible value for germination velocity being 50 (i.e. 3900/78).

Binary logistic regression was used to determine whether position, mass, maternal clone, and watering interval were predictors of germination. Cox proportional hazards regression was used to estimate the influence of position, mass, and watering interval on the rate of germination. The nonparametric Cox regression model is analogous to multiple regression and used in failure time analysis of censored data to determine whether predictor variables influence the "time until

an event occurs" (Muenchow, 1986). I defined germination as the event to calculate the hazard coefficient (Exp(β)) associated with each predictor variable (position, mass, watering interval, and the interaction of position × watering interval). I stratified data by clone, allowing me to control for potential differences among genetic individuals (genets). The ***0-d*** watering interval represented constant moisture; I tested the ***1-d*** and ***3-d*** watering interval data against the ***0-d*** data to determine whether seeds responded differently to frequent vs. intermediate watering and to frequent vs. infrequent watering. I then used separate Cox regression models, stratified by clone, to determine if position and mass predicted the rate of germination in each of the three watering intervals.

To test for effects of aging on germinability of central and peripheral seeds in the third germination study, I used logistic regression to compare the germination response of control and aged seeds. Logistic regression also was used to determine whether vernalization influenced germination in central and peripheral seeds by comparing aged seeds and vernalized seeds. I then calculated germination velocities (see above) for aged and vernalized seeds of each type to generate a measure of the speed of germination following aging and vernalization.

All data were tested for homogeneity of variances and normality prior to analysis; log transformations were used to restore homogeneity and normality where needed. Proportion data were arcsine square root transformed. All analyses were performed using SPSS version 19.0 (IBM Corp., Somers, NY, USA).

RESULTS

Genetic identities

The polymorphic loci yielded by the single selective primer pair differentiated all 50 presumed clones. Based on the 33 polymorphic loci generated, all 50 presumed clones separated by 1 m in my 50 m × 25 m plot had unique genotypes differing by 4-20 polymorphic loci. This indicated that all 50 clones were distinct genetic individuals; the term "genet" is used hereafter to refer to the 50 distinct genetic individuals. Flowering stems of the same genet were identical at all 33 polymorphic loci for 21 of the 22 genets tested, and are thus considered ramets of the same genet. The error in identifying ramets of a genet was 1 in 63 ramet samples (1.6%).

Seed morphology and mass

I observed no consistent morphological differences between central and peripheral seeds of *Packera tomentosa*. Both seed types had a similar off-white pappus (Fig. 2) and ranged in color from dark brown to reddish brown; peripheral seeds occasionally were green (Fig. 4). I observed germination in seeds of all colors. Peripheral seeds appeared slightly more curvilinear than central seeds (Fig. 4), presumably due to their location around the periphery of the composite head.

Seed mass was highly variable in this *Packera tomentosa* population (CV = 25%, N = 767). Significant variation in mass occurred between positions ($F_{1, 734.7} = 48.88$, $P < 0.001$), among genets ($F_{9, 18.9} = 4.27$, $P < 0.01$), and among ramets of the same genet ($F_{20, 182.8} = 4.24$, $P < 0.001$, Table 1). Based on partial *eta* square values, variation among genets and among ramets within a genet accounted for more variance than did variation between positions and among heads of the same ramet (Table 1). Seed mass ranged from 0.1818-0.5395 mg in central seeds

Fig. 4. Central (top row) and peripheral (bottom row) seeds of *Packera tomentosa* with pappus removed.

Table 1. Mixed-model nested ANOVA for log transformed *Packera tomentosa* seed mass data, with position treated as a fixed factor and genet, ramet nested within genet, and head nested within ramet treated as random factors. Position, Ramet (Genet), and Head (Ramet) were tested against the mean squares error (MS_{error}). Genet was tested against $MS_{Ramet(Genet)}$.

Source	d.f.	MS	*F*	Partial *eta* squared
Position	1	0.54	48.88^{***}	0.062
Genet	9	0.21	4.27^{**}	0.671
Ramet (Genet)	20	0.05	4.24^{***}	0.317
Head (Ramet)	6	0.02	1.52	0.012
Error	730	0.01	-	-

Level of significance: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

and 0.1147-0.5940 mg in peripheral seeds (Fig. 5). Central seeds were approximately 11% heavier than peripheral seeds ($\bar{x}$ ± SE, 0.3599 ± 0.00365 vs. 0.3252 ± 0.00496 mg, respectively; Table 1, 2). Peripheral seeds showed greater mass allocation to the pericarp than central seeds (69.8 ± 3.5 vs. 52.6 ± 2.6%, respectively; $t_{52.6} = 3.94$, $P < 0.001$; Table 2), but pericarp mass did not differ between positions (0.1974 ± 0.01011 vs. 0.2068 ± 0.11301 mg, respectively; $t_{57.3} = 0.622$, $P = 0.537$). In contrast, central seeds showed greater mass allocation to the embryo than peripheral seeds (47.4 ± 0.03 vs. 30.3 ± 0.03%, respectively; $t_{52.6} = 3.94$, $P < 0.001$). The embryos of central seeds were 79.5% heavier on average than those of peripheral seeds (0.1851 ± 0.01235 vs. 0.1031 ± 0.01530 mg, respectively; $t_{55.5} = 4.17$, $P < 0.001$; Table 2).

Differences in germination behavior

In the first germination study, central seeds germinated almost 4 d faster on average than peripheral seeds ($\bar{x}$ ± SE, 32.8 ± 1.51 vs. 36.2 ± 1.98 d, respectively; Table 2, 3) and at a greater proportion than did peripheral seeds (74.5 ± 3.5 vs. 36.0 ± 3.3%, respectively; Table 2, 3). Genet did not influence the average number of days required for germination or percent germination (Table 3). A significant interaction between position and genet was observed for days required and percent germination (Table 3), suggesting that the effect of position on germination varies among genetic individuals. On average, seeds of *Packera tomentosa* were highly germinable after 1-1.5 mo of dry storage at room temperature (55 ± 3.2% for all seeds).

In the second germination study, central and peripheral seeds again showed differences in germinability. Logistic regression indicated that all four variables (position, genet, mass,

Fig. 5. Seed mass frequency polygons for central seeds derived from disc florets (solid line, N = 393) and peripheral seeds derived from ray florets (broken line, N=374) of *Packera tomentosa*.

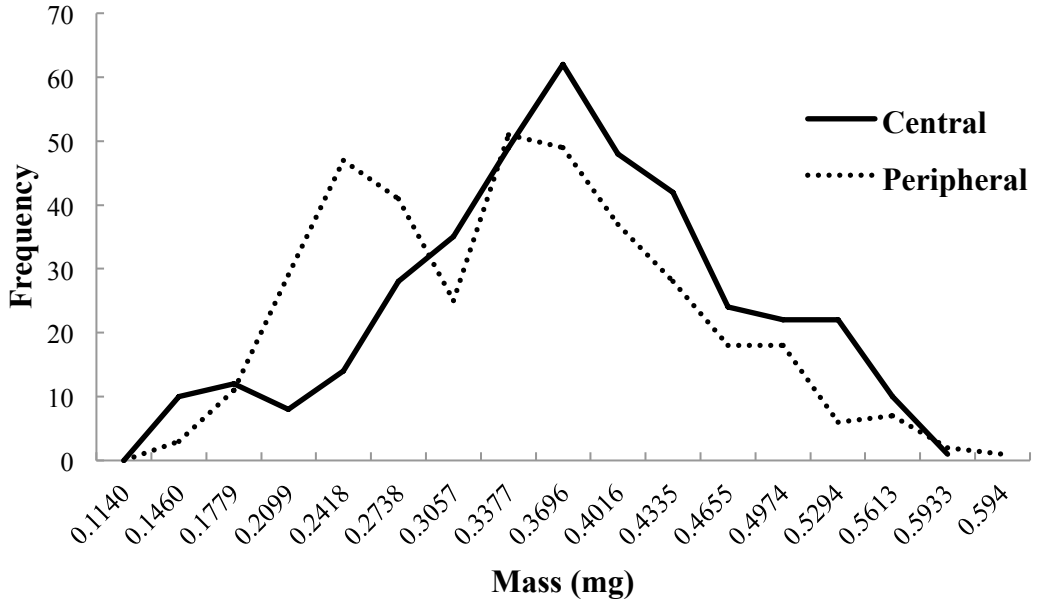


Table 2. Summary of descriptive statistics for seed traits and behaviors associated with the expression of cryptic seed heteromorphism in *Packera tomentosa* (mean ± SE (N)). Total mass, days to germination, and percent germination were compared between central and peripheral seeds using ANOVA. Mass allocation patterns were compared using Welch's t-tests.

Trait or Behavior	Central	Peripheral
Total mass (mg)***	0.3599 ± 0.00365 (393)	0.3252 ± 0.00496 (374)
Pericarp proportion (%)***	52.6 ± 2.6 (30)	69.8 ± 3.5 (30)
Pericarp mass (mg)	0.1974 ± 0.01011 (30)	0.2068 ± 0.11301 (30)
Embryo proportion (%)***	47.4 ± 0.03 (30)	30.3 ± 0.03 (30)
Embryo mass (mg) ***	0.1851 ± 0.01235 (30)	0.1031 ± 0.01530 (30)
Days to germination (d) *	32.8 ± 1.51 (40)	36.2 ± 1.98 (36)
Percent germination (%)**	74.5 ± 3.5 (40)	36.0 ± 3.3 (39)

Level of significance: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

Table 3. ANOVA comparing germinability and days to germination between central and peripheral seeds and among genets of *Packera tomentosa*.

Source	d.f.	MS	*F*
Percent Germination			
Position	1	2.68	18.31**
Genet	7	0.14	0.83
Position × Genet	7	0.17	2.20*
Error	63	0.08	-
Days Required			
Position	1	0.63	5.79*
Genet	7	0.45	3.51
Position × Genet	7	0.13	2.34*
Error	60	0.06	-

Level of significance: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

watering interval) influenced germinability (Table 4). Overall, central seeds were approximately 3.4 times more likely to germinate than peripheral seeds when mass, genet, and watering interval were constant (Table 4). Final germination percentages in the ***0-d***, ***1-d***, and ***3-d*** intervals for central seeds were 69%, 63%, and 25%, respectively, compared to 31%, 60%, and 8% for peripheral seeds (Fig. 6).

Again, I found that central seeds germinated faster than peripheral seeds. Germination velocities for central vs. peripheral seeds were 11.70 vs. 7.10, 10.23 vs. 11.54, and 5.21 vs. 1.36 in the ***0-d***, ***1-d***, and ***3-d*** frequencies, respectively. Position, mass, and watering interval all were significant predictors of the rate of germination (Table 5). A significant interaction between position and watering interval indicated central and peripheral seeds responded differently to varying moisture conditions (Table 5). Overall, central seeds germinated at a rate approximately 2.4 times faster than peripheral seeds (Table 5). The rates of overall germination in both the ***1-d*** and ***3-d*** watering intervals differed from the ***0-d*** interval (Table 5). Central seeds germinated roughly 2.2 times faster than peripheral seeds in the ***0-d*** interval and approximately 4.2 times faster in the ***3-d*** interval (Table 5). Germination in the ***1-d*** interval did not differ between positions (Table 5). Mass influenced the rate of germination in the ***0-d*** and ***1-d*** watering intervals but not in the ***3-d*** interval (Table 5).

For the third germination study, neither aging (Wald = 0.19, d.f. = 1, $P = 0.665$) nor vernalization (Wald = 0.40, d.f. = 1, $P = 0.530$) influenced overall germinability of *P. tomentosa* seeds (Fig. 7). Additionally, central and peripheral seeds did not differ in response to either the aging (Wald = 0.10, d.f. = 1, $P = 0.761$, Fig. 7) or vernalization treatment (Wald= 0.01, d.f. = 1, $P = 0.941$, Fig. 7). Final germination percentages for central vs. peripheral seeds were 68.6% vs.

Table 4. Logistic regression of the effects of position, mass, genet, and watering interval on seed germinability in *Packera tomentosa*. Exp(β) is not presented for genet or watering interval; categorical variables with more than two groups generate a separate Exp(β) for each contrast.

Source	d.f.	Wald	Exp(β)
Position	1	13.15^{***}	3.40
Mass	1	35.61^{***}	6.15
Genet	11	41.02^{***}	-
Watering interval	2	36.58^{***}	-

Level of significance: $^{***}P < 0.001$

Fig. 6. Cumulative percent germination for central (solid lines) and peripheral seeds (broken lines) in three watering intervals (***0-d, 1-d,*** and ***3-d***). First germination occurred at 20 d; data presented through 78 d.

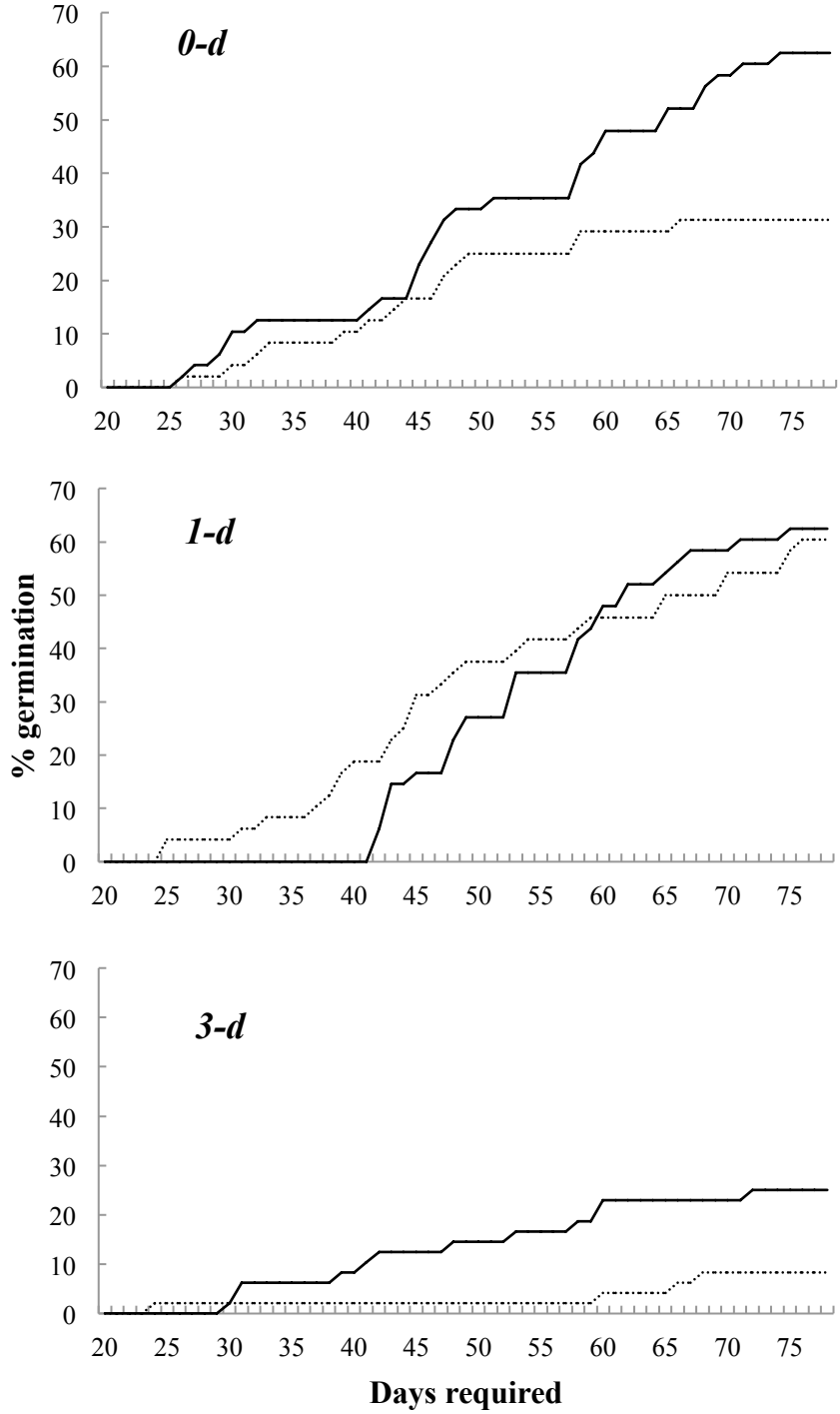
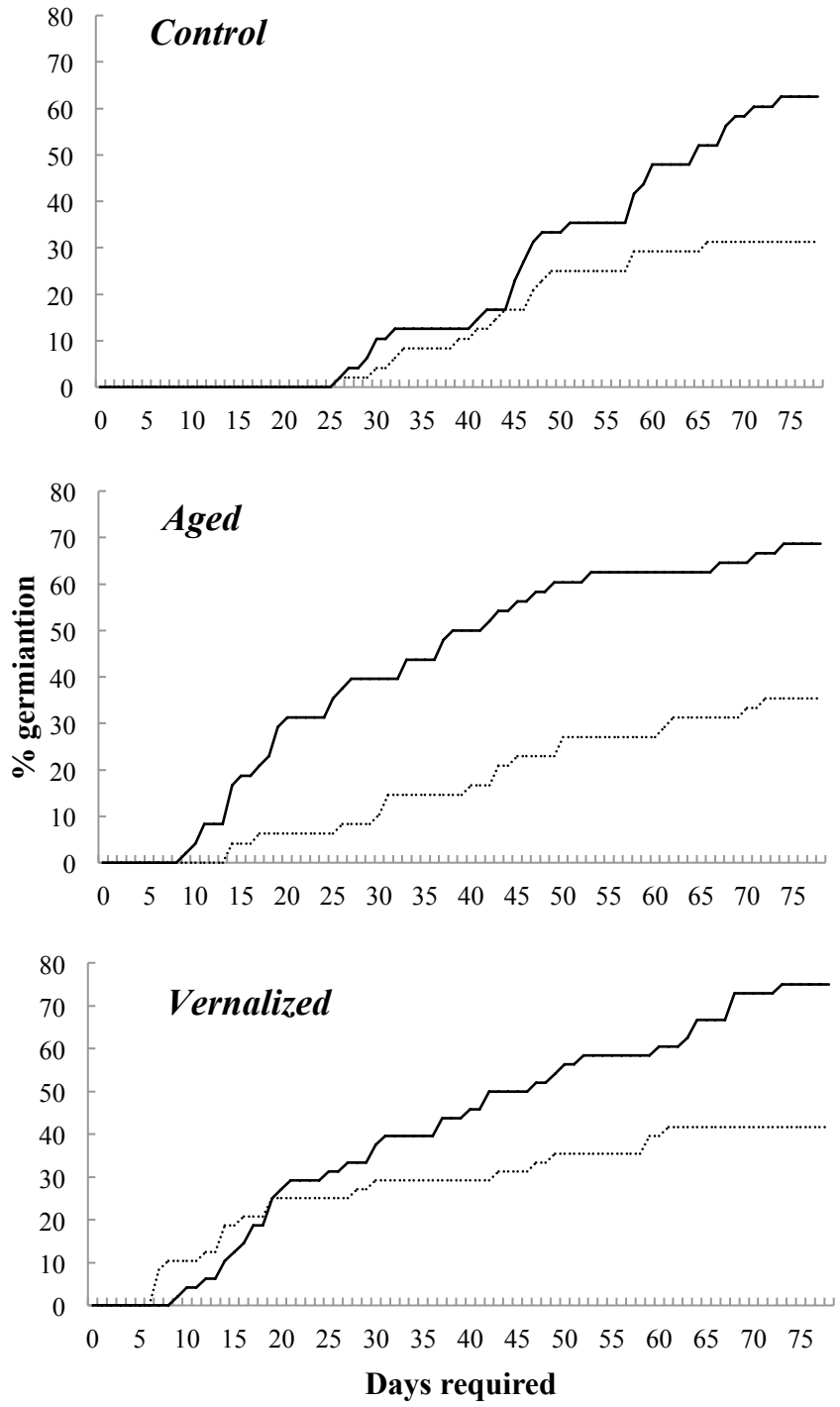


Table 5. Cox proportional hazards regressions, stratified by genet, of the effects of position, mass, and watering interval on germination rate in *Packera tomentosa*. Exp(β) is not presented for the interaction between position and watering interval because more than one contrast was generated.

Source	d.f.	Wald	Exp(β)
Pooled Data			
Position	1	7.91^{**}	2.40
Mass	1	39.82^{***}	1.96
0-d vs. ***1-d***	1	4.28^{*}	1.96
0-d vs. ***3-d***	1	8.17^{**}	0.20
Position × Watering interval	2	6.81^{*}	-
0-d *Watering Interval*			
Position	1	5.70^{*}	2.23
Mass	1	8.82^{**}	2.55
1-d *Watering Interval*			
Position	1	0.09	0.91
Mass	1	18.79^{***}	3.99
3-d *Watering Interval*			
Position	1	4.36^{*}	4.18
Mass	1	2.75	2.85

Level of significance: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

Fig. 7. Cumulative percent germination curves for control, aged, and vernalized central (solid lines) and peripheral (broken lines) seeds. Control seeds were tested for germination after ~ 1-1.5 mo of storage at room temperature; both aged and vernalized seeds were stored ~ 5 mo.



35.4% and 75.0% vs. 41.7% in response to aging and vernalization, respectively. Based on germination velocities, aging appeared to increase germination speed in central seeds, whereas vernalization may have increased germination speed in peripheral seeds (Table 6).

Table 6. Germination velocities for central and peripheral seeds in response to aging and vernalization. Germination velocity expresses germination per unit time. Velocities for seeds in the Control treatment are from the ***0-d*** watering interval (daily watering) in the second germination study.

Treatment	Central seeds	Peripheral seeds
Control	11.70	7.10
Aged	21.66	8.73
Vernalized	21.02	14.26

DISCUSSION

Packera tomentosa exhibits both continuous and discrete differences in offspring germination behavior resulting from seed mass variation and cryptic seed heteromorphism, respectively. These differences may promote seedling establishment in disturbed areas. I found that clones differed by as many as 20 of 33 (61%) polymorphic loci generated using AFLP analysis. This high genetic variation, particularly in a small (50 m × 25 m) sampling area, indicates seedling recruitment does occur in the study population of *P. tomentosa*. Aside from the position effect associated with seed heteromorphism in this species, high seed mass variation within the study population was driven by differences among and within genetic individuals. In controlled conditions, seed mass influenced the rate of germination and germinability of *P. tomentosa*, with larger seeds germinating faster and at a higher proportion. The expression of high within-population seed mass variation and a relationship between seed size and germination behavior and success have been documented in numerous plant systems (Simons and Johnston, 2006; Benard and Toft, 2007; Münzbergová and Plačková, 2010). The influence of seed size on germination in *P. tomentosa,* coupled with high seed mass variation within a genetic individual, may encourage different germination requirements among plant offspring. This spreads risk among many progeny phenotypes, potentially functioning as a bet hedging strategy in the disturbed areas where *P. tomentosa* is dominant.

Packera tomentosa produces two different types of seeds without consistent morphological differences, thus the seed heteromorphism is considered cryptic. Most Asteraceae species with seed heteromorphism produce two seed types with obvious differences in shape, color, and/or the presence of a pappus (Imbert, 2002 and references within). The production of two different seed types without obvious differences in morphology by *P. tomentosa*, a member

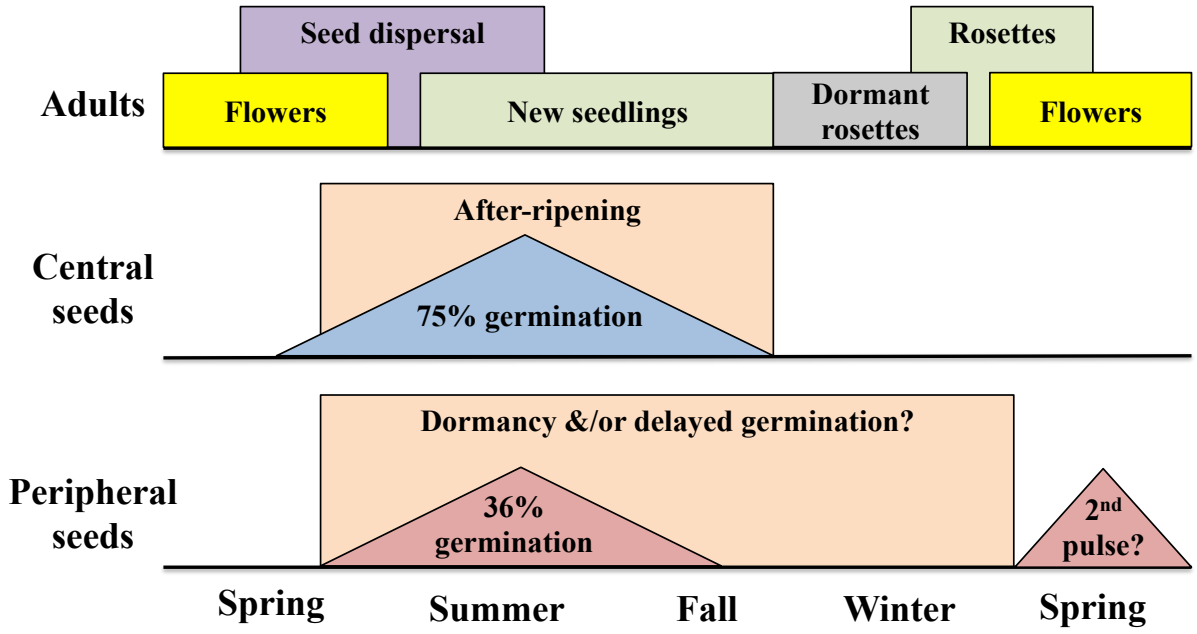
of the Asteraceae, is not surprising given the correlation between floral dimorphism and seed heteromorphism in plants (Plitmann, 1995). Although the seed types did not appear dissimilar, heteromorphism was present in *P. tomentosa* as contrasting mass characteristics and germination behavior, as documented in other taxa with cryptic seed heteromorphism (Fumanal *et al*, 2007; Pollux *et al.*, 2009). Central seeds of *P. tomentosa* were larger and had proportionally larger embryos; peripheral seeds were smaller with proportionally larger pericarps. The average pericarp mass for both seed types was the same, but embryos were larger in central seeds. This suggests that the observed differences in total mass between seed types is driven by embryo size, as is the case in other heterocarpic Asteraceae (Imbert, 2002). Because seeds of most Asteraceae species do not contain endosperm, seedling reserves reside within the embryo (Venable and Levin, 1985; Finch-Savage and Leubner-Metzger, 2006). When size differences between seed types are driven by embryo size, a difference in seedling success between the seed types may be expected (Imbert, 2002). Given the divergence in embryo size between seed types in *P. tomentosa*, central seeds probably produce larger, potentially more competitive seedlings than do peripheral seeds. Seedling traits were beyond the scope of this study; however, future work in *P. tomentosa* should address the influence of embryo size on later aspects of seedling and plant performance.

In controlled conditions, central seeds of *Packera tomentosa* germinated faster and at a higher proportion than peripheral seeds, independent of individual seed mass and maternal genet. This suggests that structural differences between seed types contributed to different germination. The divergent germination behaviors I observed between seed types could be driven by differences in pericarp features or embryo size, a pattern documented for other members of the Asteraceae (Jolls and Werner, 1989; Chmielewski, 1999; Imbert, 2002). In *P. tomentosa*, the

pericarp accounted for a significantly greater proportion of total seed mass in peripheral seeds than in central seeds. While my study focused on mass allocation and did not specifically test for differences in pericarp thickness, other work suggests the pericarp of central and peripheral seeds may have dissimilar morphologies (C.L. Jolls, unpublished results). The thickness of the fruit coat may control germination by preventing radicle protrusion and regulating water and/or gas uptake (Mohamed-Yasseen *et al.*, 1994). This decreases germination rate and percentage while increasing protection of the embryo (McEvoy, 1984). Differences in pericarp structure are responsible for divergent germination in other heterocarpic Asteraceae (e.g. *Anthemis chrysantha,* Aguado *et al*., 2011; *Heterotheca subaxillaris*, Venable and Levin, 1985; *Hemizonia increscens*, Tanowitz *et al.*, 1987). Larger embryo size could also contribute to higher, faster germination in central seeds of *P. tomentosa*. The heavier seed with the larger embryo may be expected to exhibit greater germination success since the embryo must exert sufficient pressure to fracture the seed coat and pericarp (Jones, 1978; Fenner and Thompson, 2005). The delayed germination and potential for dormancy in peripheral seeds I found in *P. tomentosa* is similar to that observed in many other heterocarpic Asteraceae, (e.g. *Bidens pilosa,* Forsyth and Brown, 1982; *Hemizonia increscens*, Tanowitz *et al*., 1987; *Heterotheca latifolia,* Venable and Levin, 1985), including its close relative *Senecio jacobaea* (McEvoy, 1984). Seeds derived from ray florets of heterocarpic Asteraceae usually exhibit lower percent germination and greater levels of dormancy than do seeds derived from disc florets (but see Brändel, 2004).

Based on the results of my germination studies, central seeds of *Packera tomentosa* have the potential to germinate quickly upon dispersal while peripheral seeds can exhibit delayed germination (Fig. 8). Statistical comparisons did not indicate differences in germinability of central and peripheral seeds when exposed to aging. There was, however, a suggestion of faster

Fig. 8. Hypothesized phenology of *Packera tomentosa* adults (top panel), central seeds (middle panel), and peripheral seeds (bottom panel) in the field over the course of five seasons, based on germination behavior observed under controlled conditions.



germination in central seeds following five months of aging. Germination reached 50% in aged central seeds 27 d sooner than in the younger central seeds. Additionally, the germination velocity of central seeds was nearly twice as high after five months of aging. Increased germination velocity with storage is a feature of seed after-ripening (Finch-Savage and Luebner-Metzger, 2006). Given this effect, as dispersed *P. tomentosa* seeds age during conditions inadequate for germination, those escaping environmental threats (e.g. seed predation, desiccation) may increase in germinability or germinate more quickly (Fig. 8). This possibly creates germinants later in the growing season, spreading seedling recruitment over time.

The vernalization of central and peripheral seeds of *Packera tomentosa* did not increase overall germinability. However, germination in peripheral seeds exposed to the vernalization treatment reached 30% germination 51 d sooner than control peripheral seeds and 47 d sooner than aged peripheral seeds. Additionally, the germination velocity of vernalized peripheral seeds was almost double that of similarly aged peripheral seeds, suggesting a faster rate of germination following cold stratification. If the low germinability I observed in peripheral seeds shortly after dispersal is due to delayed germination or dormancy, results of the vernalization study suggest that germination velocity may increase as summer temperatures cool or that dormancy may be broken at the onset of spring (Fig. 8). Asteraceae taxa generally produce seeds with no dormancy or physiological dormancy, which can be broken by after-ripening and cold stratification (Finch-Savage and Leubner-Metzger, 2006). Non-deep physiological dormancy occurs when the outer covering of a seed prevents radicle emergence (Baskin and Baskin, 1998). In *Suaeda acuminata* (Chenopodiaceae), a species with seed heteromorphism, non-deep physiological dormancy in one seed type allows for an extension of the germination period, formation of a seed bank, and the long-term recruitment of seedlings (Wang *et al*., 2012). The

pericarp of peripheral seeds of *P. tomentosa* may promote non-deep physiological dormancy and associated seed and seedling behaviors; additional germination studies are needed to test this hypothesis.

Central and peripheral seeds of *Packera tomentosa* responded differently to moisture availability. Higher, faster germination in central seeds, independent of seed mass, was observed in most of my germination studies, including those that did not manipulate water availability. Peripheral seeds germinated just as fast and at a similar percentage as central seeds, however, in the ***1-d*** watering interval designed to simulate an intermediate moisture level. The higher germination velocity for peripheral seeds exposed to intermittent watering was driven by greater initial germination than that observed for central seeds. Based on the results of this study, central seeds may have an advantage in microsites exhibiting high moisture or frequent drought. Central seeds may not, however, have a pronounced advantage in intermediate moisture levels. Increased germinability of *P. tomentosa* peripheral seeds in the ***1-d*** interval is consistent with a "priming effect", when a sufficient amount of hydration occurs to stimulate seed activation without deterioration (Hegarty, 1978; Fay and Schultz, 2009). In other words, cycles of rehydration can support higher germination than does constant moisture, a phenomenon that may be particularly important in disturbed habitats composed of microsites with varying moisture levels. In the ***1-d*** watering interval, soil moisture fluctuated slightly rather than remaining saturated or exhibiting periodic drying and re-wetting; this may increase germination success of peripheral seeds of *P. tomentosa*.

Seeds of *Packera tomentosa* appear to be intolerant of severe drought cycles, independent of position and seed mass. Germination in the ***3-d*** interval was lowest for both seed types. In some cases, periods of dehydration suppress germination or induce dormancy compared to

constant moisture conditions (Downs and Cavers, 2000). This has important implications for seedling recruitment of *P. tomentosa*, particularly in the face of global climate change. Projections of precipitation patterns suggest drought events will increase in severity and length over time (Trenberth, 2011). Soil moisture variability has greater consequences for germination success than for seedling growth in some species (Fay and Schultz, 2009), emphasizing the importance of understanding germination response to varying watering frequencies. In the field, *P. tomentosa* seeds exposed to increasing drought conditions between precipitation events may experience decreased germination success.

Both size and germination behavior of *Packera tomentosa* seeds differed among maternal genets. In other taxa, maternal nutrient levels have been demonstrated to dictate seed size (Fenner and Thompson, 2005), nutrient content (Parrish and Bazzaz, 1985), and germination success (Cheplick and Sung, 1998). Other conditions in the maternal environment, such as temperature and drought, may increase the germinability of progeny (Fenner, 1991). Additionally, maternal genetic effects can impact germination (Platenkamp and Shaw, 1993). Seed size, which may vary based on the maternal environment, is a known correlate of germination success and speed (Harper *et al.*, 1970; Simons and Johnston, 2006). While this interplay between maternal environment and seed size influences germination in some systems, the effects of maternal differences on germination were independent of seed mass in *P. tomentosa*. This suggests that while the differences in seed mass among genets could arise due to maternal microsite dissimilarity, seed mass differences alone do not explain germination dissimilarity among clones. Additional work is needed to determine if germination differences among seeds of *P. tomentosa* plants may be under the control of genetic and/or environmental maternal effects.

I suggest that investigations of species with the potential for seed heteromorphism should take into account maternal differences. Many studies of seed heteromorphism pool seeds across maternal plants prior to analysis. Thus, within- and among-individual variation in seed mass and/or germination is masked. Seed heteromorphism occurs at multiple levels, including the inflorescence, individual, clone, population, and species (Chmielewski, 1999; Matilla *et al.*, 2005). When seeds are pooled, the identification of different morphs suggests heteromorphism at the population- or species-level, but differences at the maternal- or genotype-level cannot be detected. Accounting for maternal differences associates traits of seed heteromorphism with genotypes and is necessary for our understanding of the evolution and maintenance of seed heteromorphism.

I document differences in germination behavior between seed types independent of individual seed size in *Packera tomentosa*. Most studies of seed heteromorphism focus on differences in both mass and germination behavior between seed types, but attempts generally are not made to control for the effect of seed mass differences in germination studies (but see McEvoy, 1984; van Mölken *et al*., 2005). When the seed types of heteromorphic species differ greatly in size, divergence in germination behavior could be due to seed size alone (e.g. *Tragopogon pratensis* subsp. *pratensis*, Asteraceae, van Mölken *et al*., 2005). Therefore, the failure to account for seed mass compromises our ability to discern whether differences in germination are due to dissimilarities between morphs other than seed size.

Factors contributing to differences in seed types of heterocarpic Asteraceae potentially extend beyond mass characteristics. Many taxa in this family, particularly in the Asteroideae subfamily, exhibit gynomonoecy characterized by hermaphroditic disc florets and pistillate ray florets (Bertin *et al.*, 2010). The florets can express different outcrossing rates (Cheptou *et al.*,

2001), but studies of this feature are difficult due to leaky self-incompatibility in some species (e.g. Gibson and Tomlinson, 2002; Brennan *et al.*, 2005). Given the expression of a mixed mating system with differences in outcrossing rates, ray and disc seed pools theoretically can exhibit genotypic differences (Gibson and Tomlinson, 2002). In heterocarpic Asteraceae, differences in resource allocation (e.g. sexual structures, rays) and different outcrossing rates between floret types should be joined with features of seed heteromorphism to fully understand the evolutionary ecology of this successful flowering plant family.

While suggested to be a widespread phenomenon, cryptic seed heteromorphism has been documented in few plant taxa (Silvertown, 1984; Venable, 1985a). This type of seed heteromorphism is difficult to detect since there are no obvious morphs. The Asteraceae are particularly predisposed to the production of different seed morphs, but this is commonly overlooked in germination studies because the seeds may not appear dissimilar (Chmielewski, 1999). This stresses the importance of accounting for potential differences between seeds from disc and ray florets. My study provides the first documentation of seed heteromorphism in the genus *Packera*. Other species in the genus may display germination-based heteromorphism between central and peripheral seeds as well, including 12 species considered threatened or endangered in North America (USDA, NRCS, 2012). The detection of cryptic seed heteromorphism in *Packera tomentosa*, a species producing central and peripheral seeds without obvious morphological differentiation, supports the suggestion that cryptic seed heteromorphism is more common than currently documented, particularly in the Asteraceae. This strategy may afford plant species differential germination of progeny and can support persistence in unpredictable habitats, thus contributing to the widespread success of Asteraceae taxa.

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