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Cryptic seed heteromorphism in *Packera tomentosa* (Asteraceae): differences in mass and germination

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Abstract

Seed mass variation and heteromorphism may afford plant species differential germination behavior and ultimately seedling success, particularly in disturbed habitats. We asked whether such variation occurs in *Packera tomentosa* (Michx.) C. Jeffrey (Asteraceae), a clonal species of the southeastern USA. Seed mass was compared within and among genetic individuals differentiated using amplified fragment length polymorphisms. We compared central and peripheral seeds produced by disc and ray florets, respectively, for their morphology, mass, and germination behavior, including response to water availability, aging, and cold stratification. Seed mass was highly variable both within and among individuals and influenced germination behavior. We found cryptic seed heteromorphism in *P. tomentosa*. Central and peripheral seeds had similar morphologies but dissimilar mass and biomass allocation. We used failure time analysis to detect different germination behavior. Central seeds were heavier, contained larger embryos, and germinated faster and at a higher proportion in most germination studies. Highly variable mass and heteromorphism of seeds may allow persistence of *P. tomentosa* in its disturbed habitats. Based on our results, some future studies of Asteraceae species with disc and ray florets may need to account for possible differences between seed types, even when morphological differences are not apparent. Evaluation of individual seed mass and maternal differences in germination studies may assist in the detection of cryptic seed heteromorphism, a phenomenon thought to be common, yet rarely documented.

Keywords: amplified fragment length polymorphisms (AFLP), Asteraceae, cryptic seed heteromorphism, germination, *Packera tomentosa*, seed mass.

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Introduction

The germination requirements of seeds dictate when and where plant offspring can establish. In unpredictable or heterogeneous environments, microsites available for germination vary both spatially and temporally in abiotic conditions such as light, temperature, and soil microtopography, all of which can affect seed and seedling success (Sheldon 1974). Once dispersed, seeds are exposed to fluctuating cycles of hydration and dehydration, often resulting from sporadic precipitation (Kagaya *et al*. 2005). Variability in soil features can limit plant reproductive success if all seeds require identical conditions for germination. Variation in seed size influences the success and timing of germination, seedling establishment, fecundity, and survival, and can ensure some offspring encounter favorable conditions (Simons & Johnston 2006, and references therein). When variable seed size impacts germination and this 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 plant taxa, with coefficient of variation (CV) values for seed mass commonly >5%, considered "large" for a biological trait (Thompson 1984). Variation in seed size

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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). Within an individual, seed mass can be independent of genetic differences among seeds due to parentage (Tweney & Mogie 1999).

Seed heteromorphism or heterocarpy, the production by a single plant of two or more types of diaspores with different forms and/or behaviors, may promote persistence in disturbed habitats (Venable 1985). Distinctions among seed or fruit morphs commonly include mass, color, dispersal, and germination patterns, with contrasting germination behaviors occurring in response to environmental factors such as temperature, light, and salinity (Imbert 2002). Seed heteromorphism is associated with an annual life cycle and occurrence in unpredictable habitats (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 & Koubek 2003).

Ecological and evolutionary questions related to seed heteromorphism typically are directed toward species that produce morphologically distinct seeds or fruits. Seed heteromorphism can be "cryptic", however, when seed types display different ecological behaviors unaccompanied by obvious morphological dissimilarities (Venable 1985). This type of seed heteromorphism may be overlooked due to a lack of morphological distinction among seeds and the difficulty in detecting the contrasting behaviors that define the heteromorphism (Venable 1985). In documented cases of cryptic seed heteromorphism, contrasting behaviors arise in dispersal, germination, and growth of plants derived from seed types (e.g. Fumanal *et al*. 2007; Pollux *et al*. 2009).

Although documented in many families, seed heteromorphism is most common in the Asteraceae (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 often referred to as heterocarpy (i.e. different fruits). The prevalence of heterocarpy in the Asteraceae is attributable to the anatomy of the composite head, which typically includes an outer whorl of ray florets and inner whorls of disc florets. Morphological distinctions among the fruits can include color, shape, and trichome characteristics, but most relate to size and pappus structures (Imbert 2002). Generally, two morphs are produced: central fruits derived from interior disc florets and peripheral fruits derived from outer ray florets. The central morphs commonly are smaller and more dispersive than peripheral morphs (Imbert 2002). Central morphs are heavier than peripheral morphs, however, in some heterocarpic Asteraceae (e.g. *Picris radicata*, Ellner & Shmida 1984; *Bidens pilosa*, Rocha 1996). Size differences between fruit morphs generally relate to embryo size; the larger of the two morphs also tends to contain a larger embryo.

In heterocarpic Asteraceae, the most commonly reported difference in behavior between morphs occurs in germination. The conditions required for germination usually are more restricted for one morph than the other (Venable 1985). Generally, central morphs germinate quickly while peripheral morphs show delayed germination or exhibit dormancy (Imbert 2002), allowing plants to disperse progeny in time. While continuous seed mass variation creates a range of germination behavior, seed heteromorphism involves discrete classes of seeds that can have opposing roles, such as population maintenance versus colonization. Both strategies broaden the range of conditions under which seeds can germinate and thus promote the ability to reproduce in stressful or unpredictable habitats (Fenner & Thompson 2005).

We investigated aspects of the seed ecology of *Packera tomentosa* (Michx.) C. Jeffrey (Asteraceae) (Jeffrey 1992), woolly ragwort, a perennial species in tribe Senecioneae, subtribe Senecioninae (Bain & Golden 2000; Pelser *et al*. 2007; Fig. 1a). The genus *Packera* Á. Löve and D. Löve is thought to have originated in Mexico (Bain & Golden 2000), evolving from the ancestral lineage of *Senecio* (Knox & Palmer 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 *Emilia* (Bain & 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 USA, where it inhabits open meadows, roadsides, and sandy, shallow soils (eFloras 2012). Plants (30–60+ cm tall) occur as stands of clustered rosettes of basal lanceolate-elliptic leaves, 40–120+ mm long by 20–50+ mm wide, with subentire, crenate to serrate-dentate margins, often with a floccose tomentum proximally. *Packera tomentosa* reproduces vegetatively, producing offshoots of rosettes (ramets) connected by below-ground 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. Flowering occurs from (March) May to early June (eFloras 2012). The cypselae of *P. tomentosa* are hispid and bear a calyx modified as a pappus of capillary bristles 5–7 mm long (Fig. 1b). In

Fig. 1 (a) *Packera tomentosa* flowering heads showing disc florets, which produce central seeds (C), and ray florets, which produce peripheral seeds (P) (scale bar = 5 mm). (b) Central (top row) and peripheral (bottom row) seeds of *P.tomentosa* with intact pappus (scale bar = 1 mm).

Asteraceae, the pappus and fruit coat can collectively contribute to dispersal as well as germination of the fertilized ovule, allowing the entire diaspore to function as a seed; hereafter, we use the term seed. Although the exact timing of seedling emergence in the field is currently unknown, it may occur in the fall when temperatures are cooler and rain is more abundant, at least in granitic outcrop populations (Chapman & Jones 1971).

Packera tomentosa is associated with disturbed and unpredictable habitats (eFloras 2012) 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). Many germination studies of heteromorphic seeds test for differential responses to light intensity, temperature, and/or salinity, yet fewer attempt to measure responses to moisture availability (but see Wang *et al*. 2010). We hypothesized that seed mass variation and/or heteromorphism in *P. tomentosa* may broaden the range of favorable microsites for germination, defined in part by water availability, and promote persistence in the highly disturbed habitats where this species is successful. We used seed mass characteristics and germination studies to test for seed mass variation and heterocarpy. Specifically, we 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 cold stratification influence germination in central and/or peripheral seeds?

Materials and methods

Study site, seed collection, and storage

We studied a population of *P. tomentosa* located at East Carolina University's West Research Campus (WRC), a 235 ha tract of land located northwest of Greenville, western-central Pitt County, North Carolina, USA. 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. Approximately 60% of WRC is jurisdictional wetland and the soil is generally nutrient-poor (Goodwillie & Franch 2006). *Packera 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. The density of *P. 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 variation 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. We selected distinct groups of plants that were presumed to be clones (genetic individuals) separated by at least 1 m when possible. We gently excavated the rhizome to determine whether rosettes were part of the same clonal individual (genet). Genetic identities later were analyzed using amplified fragment length polymorphisms (AFLP, see below) generated from leaf tissue collected from each of the rosettes. We collected three heads per flowering stem for 50 presumed clones. Three flowering stems per clone were sampled for 37 clones; we used one flowering stem for the remaining 13 clones for which connected ramets could not be identified with confidence. Heads were stored intact at room temperature prior to dissection, determination of seed mass, and germination experiments.

Confirmation of genetic identities

Amplified fragment length polymorphism profiles were generated using leaf tissue to confirm that the 50 presumed clones were genetically distinct. To confirm ramets of the same clone were identified correctly, we analyzed leaf tissue from ramets of 22 of the 50 clones ($n = 63$ samples). Amplified fragment length polymorphism is a rapid, PCR-based molecular tool used for genotyping or fingerprinting individuals (Vos *et al*. 1995; Meudt & 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 & Doyle (1987). We implemented an AFLP protocol adapted from that of Vos *et al*. (1995) using *Mse*I and *Eco*RI adaptors. Preamplification reactions used *Mse*I and *Eco*RI primers containing a single selective nucleotide. Final selective amplifications were performed with *Mse*I-CTG and *Eco*RI-ACC selective primers.

The AFLP profile for each sample was analyzed and scored using GeneMapper version 4.1 (Applied Biosystems, Foster City, CA, USA), with peaks (fragments) scored as either present or absent. The analysis yielded 88 polymorphic loci. We excluded ambiguous loci, narrowing the list to 33 loci that were easily resolvable. Presence/absence data were used to construct a genetic distance matrix using GenAlEx 6.41 (Peakall & Smouse 2006). We then examined these data to confirm that presumed ramets of the same clone of *P. tomentosa* indeed had identical genotypes (i.e. identical at all 33 loci) and that presumed clones had unique genotypic fingerprints (i.e. not identical at all 33 loci).

Seed mass characteristics

Five mature, filled (containing an embryo) seeds per position (central vs. peripheral) from each head were chosen randomly from 10 clones (three heads per flowering stem, three stems per clone). Eight heads did not contain filled seeds and were excluded; thus, 82 heads were included in the analysis. From some heads ($n = 17$), five filled seeds per position could not be obtained; in such cases we used two to four filled seeds. Seeds were gently squeezed with forceps to determine whether they contained embryos (Baskin & Baskin 1998); unfilled seeds were excluded from the analysis. We weighed each seed individually without the pappus to the nearest 0.0001 mg (Cahn model E-15, Cerritos, CA, USA). Seed mass variation was analyzed using a mixed-model nested ANOVA, treating "Position" as a fixed effect and all other levels as random effects. Partial eta-square values were used to compare the relative amount of variance explained by each level when considered alone. In the model, all levels were tested against the mean squares error term (MS_{error}) except "Genet" (variation among clones), which was tested against the mean squares of its subsequent level.

To determine whether central and peripheral seeds differed in mass allocation, we dissected 30 seeds into pericarp and embryo. Pericarps were weighed individually to the nearest 0.0001 mg. Embryo mass was inferred from these measurements [(total mass) – (pericarp mass)]. We determined the proportion of total mass allocated to each structure [(pericarp mass)/(total mass) and (embryo mass)/(total mass)]. Comparisons of mass allocation between seed types were performed using Welch's *t*-tests.

Germination of central versus peripheral seeds

An initial study was conducted to gather basic germination data for central and peripheral seeds of *P. tomentosa*, asking whether position and/or maternal clone (genet) influence percent germination and number of days to germination. Only seeds containing an embryo, determined using squeezing, were used for all germination studies. Seeds had been stored dry at room temperature for approximately 1–1.5 months when germination trials began. Seeds of the same position and maternal clone ($n = 8$ clones) were placed in Petri dishes on autoclaved sand in groups of five ($n = 40$ and 39 dishes of central and peripheral seeds, respectively). Seeds were grouped by maternal clone to control for potential differences among genetic individuals; the number of replicate dishes per clone ranged from two to 10. Seeds of *P. tomentosa* require at least 12 h of light for germination (Chapman & Jones 1971). Seeds were exposed to 16 h light (25°C) and 8 h dark (15°C) per day at 40–45% relative humidity for 70 days, at which point no germination had occurred for 7 days (Percival Scientific, Model AR-41 L3, Perry, IA, USA). We chose a 25/15°C temperature regime to simulate field temperatures encountered by seeds in this population within two or three months following dispersal. Seeds were watered and checked daily for emergence of the radicle, our criterion for germination. We did not attempt to determine whether ungerminated seeds were nonviable. Comparisons between seed types of percent germination and the number of days to germination were performed using ANOVA, treating "Position" as a fixed effect and "Genet" as a random effect.

Influence of position, clone, mass, and watering interval on germination

We conducted a second germination study using seeds of known weight to determine whether position, maternal

clone, and/or individual seed mass affect germination in *P. tomentosa*. We also asked whether central and peripheral seeds respond differently to frequent, intermediate, and infrequent watering intervals, in percent germination and the rate of germination. For this study, seeds had been stored dry at room temperature for approximately 1–1.5 months. We used central and peripheral seeds from 12 randomly selected clones. The germination protocol was identical to the study above, but with four seeds per dish and a study duration of 78 days, at which point no new germination had occurred for 7 days. 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 watering intervals based on the number of days between watering events: 0 days (*0-d*, frequent watering), 1 day (*1-d*, intermediate watering), and 3 days (*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.

We determined germination velocity for each group (each of the two seed positions in each of the three watering intervals). Germination velocity, an index of germination speed allowing comparison among studies, was calculated as $\sum G/t$, with G being the cumulative percent germination at 2-day intervals and t the total number of days sampled (Khan & Ungar 1997). Higher index values represent more rapid germination, with a maximum value of 50 (in our study, 3900/78). We determined whether position, mass, maternal clone, and watering interval predicted germination using binary logistic regression; the use of maternal clone as a predictor variable allowed us to control for differences among genetic individuals. Cox proportional hazards regression was used to estimate the influence of position, mass, and watering interval on the rate at which germination occurred. The nonparametric Cox regression model is comparable to multiple regression and used in failure time analysis of censored data (i.e. seeds which have not germinated by the end of the study) to determine whether predictor variables influence the "time until an event occurs" (Muenchow 1986). This type of analysis has important advantages over other methods for the treatment of germination data, and provides the opportunity to determine whether factors predict both germinability and the rate or speed of germination while accounting for seeds that did not germinate by the end of the study (Onofri *et al*. 2010; McNair *et al*. 2012). The model defined germination as the event and calculated the hazard coefficient (Exp(β)) associated with each predictor variable (position, mass, watering interval, and the interaction of position × watering interval). In the Cox model, we stratified data by clone, allowing us to control for potential differences in germination among genetic individuals. In this analysis the *0-d* watering interval represented constant moisture; we tested the *1-d* and *3-d* data against the *0-d* data to determine whether seeds responded differently to frequent versus intermediate watering and to frequent versus infrequent watering. We 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.

Influence of seed aging and cold stratification on germination

Beginning late September 2011, *P. tomentosa* seeds were moist-stratified at 2°C for 30 days ("cold stratified" seeds) to determine whether seeds were dormant at time of dispersal. Cold stratification is often used to break seed dormancy (Baskin & Baskin 1998). We observed individual seeds from six clones for germination ($n = 96$ seeds, 48 each central and peripheral). To ensure any effects on germination behavior in this study were due to cold stratification and not simply aging, we compared them to a matched complement of 96 seeds (48 each central and peripheral) that had been stored dry at room temperature instead of cold stratified ("aged" seeds). 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 ("control" seeds). Cold stratified and aged seeds were used from the same six clones and germinated simultaneously. Temperature, photoperiod, and relative humidity for this study were the same as in those described above. Seeds were watered and checked for emergence of the radicle daily until no further germination had occurred for 1 week (78 days). To test for effects of aging on overall germinability, we compared the germination response of control and aged seeds using binary logistic regression. Logistic regression also was used to determine whether cold stratification influenced overall germination by comparing aged seeds and cold stratified seeds. For both tests, an interaction variable was created to determine whether central and peripheral seeds responded differently to each treatment (aging or cold stratification). We then calculated germination velocities (see above) for aged and cold stratified seeds of each type.

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

Results

Genetic identities

Based on our AFLP analysis, all 50 presumed clones separated by 1 m in our 50 m × 25 m plot had unique genotypes differing by 4–20 of the 33 (12–61%) polymorphic loci we compared. This indicated that all 50 clones were distinct genetic individuals (genets). Flowering stems of the same genet were identical at all 33 polymorphic loci for 21 of the 22 genets tested, and were thus considered ramets of the same genet. The error in identifying ramets of a genet in the field was 1.6% (1 in 63 samples).

Seed morphology and mass

We observed no qualitative morphological differences between central and peripheral seeds of *P. tomentosa*. Both seed types had a similar off-white pappus (Fig. 1b) and ranged in color from dark brown to reddish brown; peripheral seeds occasionally were green. We observed germination in seeds of all colors. Peripheral seeds appeared slightly more curvilinear than central seeds, presumably due to their location around the periphery of the composite head.

Seed mass was highly variable in this *P. tomentosa* population (CV = 25%, $n = 767$). Significant variation in mass was detected between positions, among genets, and among ramets of the same genet (Table 1). Partial eta-square values indicated that variation among genets and among ramets within a genet accounted for more variance of seed mass than did variation between positions and among heads of the same ramet (Table 1). Seed mass ranged from 0.1818 to 0.5395 mg in central seeds and 0.1147 to 0.5940 mg in peripheral seeds. On average, central seeds were approximately 11% heavier than peripheral seeds (Table 2). Peripheral seeds showed greater mass allocation to the pericarp than central seeds ($t_{52.6} = 3.940$, $P < 0.001$), but pericarp mass did not differ between positions ($t_{57.3} = 0.622$, $P > 0.05$; Table 2). Central seeds showed greater mass allocation to the embryo than peripheral seeds (Table 2), with embryos of central seeds 79.5% heavier on average than those of peripheral seeds ($t_{55.5} = 4.170$, $P < 0.001$; Table 2).

Table 1 Seed mass variation in *Packera tomentosa*, with "Position" treated as a fixed factor and all other levels treated as random factors. All levels were tested against the mean squares error (MS_{error}) except "Genet", which was tested against $MS_{Ramet(Genet)}$

Variable	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	—	—

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Differences in germination behavior

In our germination study comparing central and peripheral seeds, central seeds germinated almost 4 days faster on average than peripheral seeds ($F_{1,60} = 5.786$, $P < 0.01$; Table 2) and at a greater proportion than did peripheral seeds ($F_{1,63} = 18.305$, $P < 0.01$; Table 2). Germination responses did not differ among genetic individuals as measured by the number of days to germination ($F_{7,60} = 3.514$, $P > 0.05$) and percent germination ($F_{7,63} = 0.826$, $P > 0.05$). A significant interaction between position and genet observed for days to germination ($F_{7,60} = 2.341$, $P < 0.05$) and percent germination ($F_{7,63} = 2.203$, $P < 0.05$) suggested that differences in germination behaviors between seed types may vary among genetic individuals (maternal clones). Overall, seeds of *P. tomentosa* were highly germinable (55.0 ± 3.2%).

Our study manipulating watering frequency showed that germinability also was influenced by position (logistic regression $Wald = 13.149$, d.f. = 1, $P < 0.001$), maternal genet ($Wald = 41.022$, d.f. = 11, $P < 0.001$), individual seed mass ($Wald = 35.607$, d.f. = 1, $P < 0.001$), and watering interval ($Wald = 36.575$, d.f. = 2, $P < 0.001$). Germination first occurred at approximately 25 days in all three watering intervals (Fig. 2). Central seeds were approximately 3.4 times more likely to germinate than peripheral seeds

Table 2 Comparisons of traits and behaviors between central and peripheral seeds associated with the expression of cryptic seed heteromorphism in *Packera tomentosa* ($\bar{x} \pm SE\,(n)$). Total mass, percent germination, and days to germination were compared using ANOVA. All mass allocation comparisons were made using Welch's *t*-tests

Trait or behavior	Central seeds	Peripheral seeds
Total mass (mg)***	0.3599 ± 0.0037 (393)	0.3252 ± 0.0050 (374)
Pericarp proportion (%)***	52.6 ± 2.6 (30)	69.8 ± 3.5 (30)
Pericarp mass (mg)	0.1974 ± 0.0101 (30)	0.2068 ± 0.1130 (30)
Embryo proportion (%)***	47.4 ± 0.03 (30)	30.2 ± 0.03 (30)
Embryo mass (mg)***	0.1851 ± 0.0124 (30)	0.1031 ± 0.0153 (30)
Germination (%)**	74.5 ± 3.5 (40)	36.0 ± 3.3 (39)
Days to germination*	32.8 ± 1.5 (40)	36.2 ± 2.0 (36)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

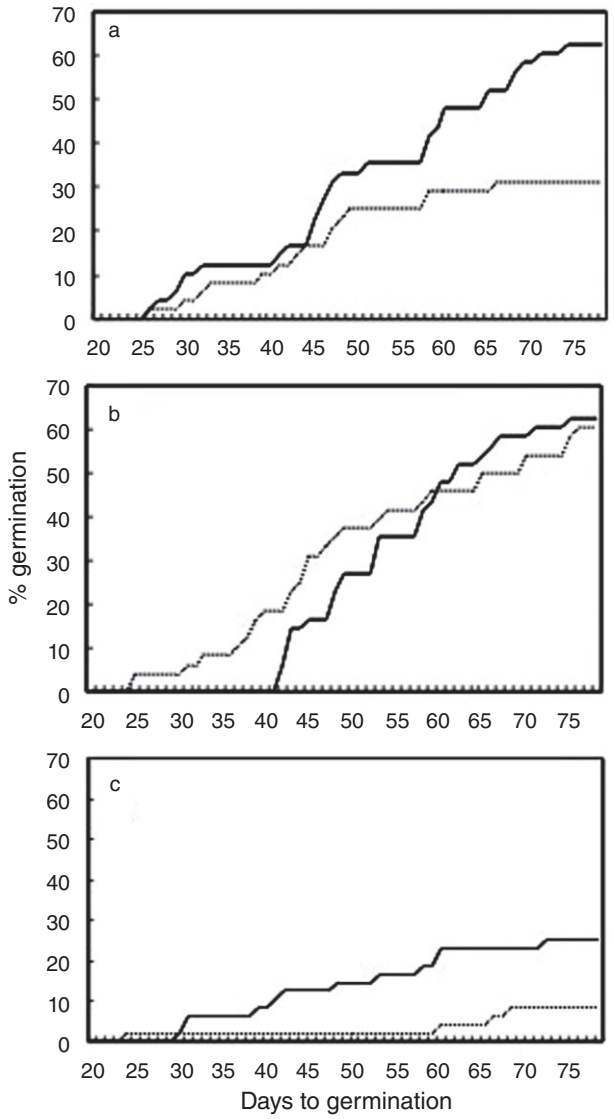

Fig. 2 Germination curves for central seeds (solid lines) and peripheral seeds (broken lines) of *Packera tomentosa* in (a) frequent, (b) intermediate, and (c) infrequent watering intervals. First germination occurred at 20 days; data presented through 78 days.

when mass, genet, and watering interval were held constant (Exp(β) = 3.396). Cumulative 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. 2).

Position, mass, and watering interval also influenced of the rate of germination (Table 3). Germination velocities for central versus peripheral seeds were 11.70 versus 7.10, 10.23 versus 11.54, and 5.21 versus 1.36 in the *0-d*, *1-d*, and *3-d* frequencies, respectively, bearing in mind that higher values indicate faster germination. A significant interaction between position and watering interval indicated central and peripheral seeds responded differently to varying moisture conditions (Table 3). Overall, central seeds germinated about 2.4 times faster than peripheral seeds (exp(β) = 2.403). The rates of overall germination in both the *1-d* and *3-d* watering intervals differed from the *0-d* interval (Table 3). Germination rate in the *1-d* interval did not differ between positions (Table 3). Mass influenced the rate of germination in the *0-d* and *1-d* watering intervals but not in the *3-d* interval (Table 3).

Neither aging (*Wald* = 0.187, d.f. = 1, $P > 0.05$) nor cold stratification (*Wald* = 0.395, d.f. = 1, $P > 0.05$) influenced overall germinability of *P. tomentosa* seeds. The first day of germination occurred sooner after aging (9 days) and cold stratification (7 days) compared to 25 days for the control (Fig. 3). The average time to germination was 33.9 days for aged seeds and 32.3 days for cold stratified seeds, compared to 49.3 days for control seeds, suggesting additional aging promoted quicker germination. Central and peripheral seeds did not differ in response to either the aging (*Wald* = 0.092, d.f. = 1, $P > 0.05$) or cold stratification treatment (*Wald* = 0.005, d.f. = 1, $P > 0.05$). Again, central seeds germinated to a higher percentage than did peripherals: 68.6% versus 35.4% and 75.0% versus 41.7% in response to aging and cold stratification, respectively (Fig. 3). Faster germination with aging by central seeds is reflected in the higher germination velocities: 11.70, 21.66, and 21.02 in the control, aged, and cold stratification treatments, respectively. Peripheral seeds were still slower to germinate, however, appearing only to respond to cold stratification; germination velocities in the control, aged, and cold stratification treatments were 7.10, 8.73, and 14.26, respectively.

Discussion

Cryptic seed heteromorphism in Packera tomentosa

Packera tomentosa seeds exhibit both continuous and discrete differences in germination behavior resulting from seed mass variation and cryptic seed heteromorphism, respectively. It has been suggested previously that most recruitment for this species comes from rhizomes rather than seeds (Chapman & Jones 1971). We found that clones differed by as many as 61% of the polymorphic loci generated using AFLP. This high amount of genetic variation, particularly in the relatively small area we sampled, indicates seedling recruitment does occur in *P. tomentosa*, at least in our study population. A detection of high within-population seed mass variation and a relationship between seed size and germination, found here in *P. tomentosa*, also has been documented in other plant systems (Benard & Toft 2007; Münzbergová & Plačková 2010). In *P. tomentosa*, the influence of seed mass on germination, coupled with high seed mass variation within a genetic

Interval	Variable	β	SE of β	Wald	Exp(β)
Pooled data	Position**	0.877	0.312	7.910	2.403
	Mass***	0.671	0.106	39.821	1.956
	0-d vs. *1-d* *	0.672	0.325	4.280	1.959
	0-d vs. *3-d* **	–1.609	0.563	8.168	0.200
	Position × watering interval*	—	—	6.808	—
0-d	Position*	0.800	0.335	5.695	2.226
	Mass**	0.935	0.315	8.823	2.548
1-d	Position	–0.110	0.289	0.144	0.896
	Mass***	1.224	0.303	16.290	3.401
3-d	Position*	1.430	0.685	4.357	4.177
	Mass	1.046	0.630	2.753	2.845

Table 3 The effects of position, mass, and watering interval on the rate of germination of *Packera tomentosa* seeds using Cox proportional hazards regression analysis. For each analysis data were stratified by maternal clone

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

individual, may diversify germination requirements among plant offspring. Provided such variation translates into differences in germination behavior in the field, this can spread risk among many progeny phenotypes, potentially functioning as a bet-hedging strategy in the disturbed areas where *P. tomentosa* is dominant.

Packera tomentosa expresses cryptic seed heteromorphism through the production of morphologically similar seed types (Fig. 1b) with contrasting size and germination behavior (Table 2). The observed differences in total mass between seed types appears to be driven by embryo size, as is the case in other heterocarpic Asteraceae (Imbert 2002). Seeds of most Asteraceae species do not contain endosperm and seedling reserves reside within the embryo (Venable & Levin 1985; Finch-Savage & Leubner-Metzger 2006). When one seed type produces a proportionally larger embryo, a difference in seedling size may be expected (Venable & Levin 1985). 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.

Seed heteromorphism characterized by dissimilar germination behavior

Asteraceae generally produce seeds with either no dormancy or physiological dormancy, which can be broken by after-ripening and cold stratification (Finch-Savage & Leubner-Metzger 2006). While our work did not specifically test for it, *P. tomentosa* seeds, both central and peripheral, may require after-ripening after dispersal, corroborating suggestions that aging is necessary for germination in this species (Chapman & Jones 1971). We found relatively high germination in seeds stored for 1–1.5 months, consistent with the need for an aging period, albeit brief. Additionally, although seeds subjected to further aging and cold stratification treatments germinated at percentages similar to control seeds (Fig. 3), germination occurred sooner than in younger seeds. Our work shows central and peripheral seeds differ in germination behavior, including responses to aging, cold stratification, and water availability, and suggests that a physiological mechanism may be influencing differences in germination.

In controlled conditions, central seeds of *P. tomentosa* germinated faster and at a higher percentage than peripheral seeds, independent of individual seed mass and maternal genet. This suggests that structural differences between seed types contributed to distinct germination behaviors. This distinction could be driven by differences in pericarp features or embryo size, patterns documented in other Asteraceae (Jolls & Werner 1989; Chmielewski 1999). In *P. tomentosa*, the pericarp accounted for a significantly greater proportion of total seed mass in peripheral seeds than in central seeds. While our study did not specifically test for differences in pericarp thickness, other work on *P. tomentosa* suggests this trait may differ between central and peripheral seeds (C.L. Jolls, unpubl. res.). The thickness of the fruit coat can control germination by preventing radicle protrusion and regulating water and/or gas uptake (Mohamed-Yasseen *et al*. 1994), thus decreasing germination rate and percentage while increasing protection of the embryo (McEvoy 1984). Differences in pericarp structure are responsible for divergent germination between seed types in other heterocarpic Asteraceae (e.g. *Anthemis chrysantha*, *Aguado et al*. 2011; *Heterotheca subaxillaris*, Venable & Levin 1985). Larger embryo size also could explain 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).

Central seeds of *P. tomentosa* may have the potential to germinate quickly upon dispersal while germination of peripheral seeds is delayed. Although the dissimilarity in

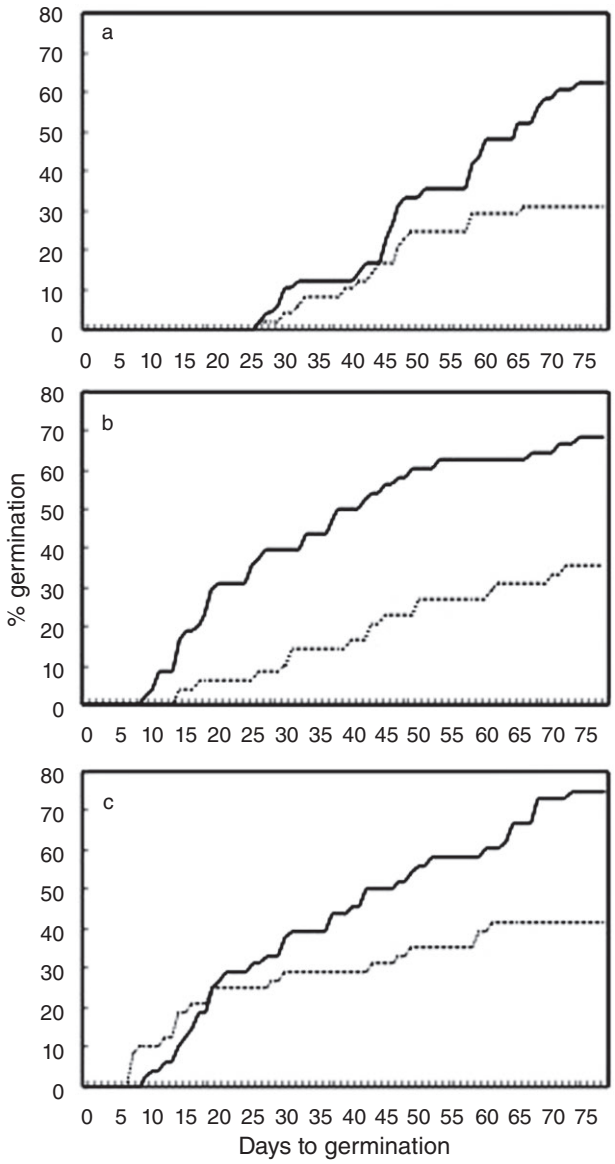

Fig. 3 Germination curves for central seeds (solid lines) and peripheral seeds (broken lines) of *Packera tomentosa* in response to (a) control, (b) aging, and (c) cold stratification treatments.

mean germination time between central and peripheral seeds is modest (Table 2), even a few days' difference in germination can produce differences in survival due to seedling developmental stage, depending on temporal variation in the environment, for example drought (Harper 1977). In our study of the effects of aging and cold stratification, germination velocity increased in central seeds following 5 months of aging, consistent with seed after-ripening (Finch-Savage & Leubner-Metzger 2006). Given this effect, as *P. tomentosa* central seeds age during conditions inadequate for germination, those escaping environmental threats (e.g. seed predation, desiccation) may increase in germinability or germinate more quickly if favorable conditions arise. This could create germinants later in the growing season, spreading seedling recruitment through time.

In our study of the effects of aging and cold stratification on germination, the germination velocity of cold stratified peripheral seeds was almost double that of similarly aged peripheral seeds, indicating a faster rate of germination following cold stratification. This suggests cooling summer temperatures may stimulate germination of peripheral seeds or that dormancy in these seeds may be broken during the fall or at the onset of the following spring. The expression of dormancy in one seed type of plants with seed heteromorphism can allow for an extension of the germination period, formation of a seed bank, and the long-term recruitment of seedlings (Wang *et al*. 2012). In *P. tomentosa*, the pericarp of peripheral seeds may promote physiological dormancy and associated seed and seedling behaviors; additional studies are needed to test this hypothesis.

Differences in germination behavior between seed types of *P. tomentosa* are responses, in part, to moisture availability. Higher, faster germination in central seeds, independent of seed mass, was observed in all our controlled experiments except for the one simulating intermediate watering conditions (*1-d*). The higher germination velocity for peripheral seeds exposed to intermittent watering was driven by greater initial germination than that observed for central seeds. Our watering interval study suggests that central seeds may have an advantage in microsites exhibiting high or low moisture availability, but not under intermediate moisture levels. Increased germination success of *P. tomentosa* peripheral seeds in the intermediate watering interval is consistent with a "priming effect", in which a sufficient amount of hydration occurs to stimulate seed activation without deterioration (Hegarty 1978; Fay & 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 with microsites having varying moisture levels.

Seeds of *P. tomentosa* showed reduced germination in response to severe drought cycles (infrequent watering intervals, Fig. 2). This is consistent with results from studies of other species in which periods of dehydration suppress germination compared to constant moisture conditions (e.g. Downs & Cavers 2000). The potential for drought-induced changes in *P. tomentosa* germination patterns to alter seedling emergence, success, and ultimately recruitment in the field merits future research, particularly in the face of global climate change and projections of increasingly severe drought events (Trenberth 2011; Walck *et al*. 2011).

Both the size and germination behavior of *P. tomentosa* seeds differed among maternal genets. Seed size, which may influence aspects of germination, can differ among plants due to maternal nutrient levels (Fenner & Thompson 2005). Germination behavior also may be influenced by other features of the maternal environment (temperature and drought; Fenner 1991), or by maternal genetic effects (Platenkamp & Shaw 1993; Kagaya *et al*. 2011). While this interplay between maternal effects and seed size influences germination in some systems, the effects of maternal differences on germination were independent of seed mass in *P. tomentosa* (as indicated by our logistic regression analysis of response to watering frequency). 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 contrasting germination 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.

Overcoming challenges associated with the detection of cryptic seed heteromorphism

We suggest that investigations of species with the potential for seed heteromorphism should take into account differences among maternal plants. Seed heteromorphism occurs at multiple levels, including the inflorescence, individual, clone, population, and species (Chmielewski 1999; Matilla *et al*. 2005). Many studies of seed heteromorphism pool seeds across maternal plants prior to analysis, masking within- and among-individual variation in seed mass and/or germination. When seeds are pooled, the identification of different morphs can suggest heteromorphism at the population or species level, but differences at the maternal- or genotype-level cannot be detected. Cryptic seed heteromorphism is a species-level trait, expressed at the level of the individual genotype that can be detected only against the background of seed variation within and among individual plants. 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.

We document cryptic heteromorphism in *P. tomentosa* as differences in germination behavior between seed types independent of individual seed size. Most studies of seed heteromorphism focus on differences in both mass and germination behavior between seed types, but generally, attempts 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 beyond 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. 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 & 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 family.

Conclusions

Many of the Asteraceae are predisposed to the production of different seed morphs, but this is commonly overlooked in germination studies because the seeds do not appear dissimilar (Chmielewski 1999). Our study highlights the importance of accounting for potential differences between seeds from disc and ray florets. We provide the first documentation of seed heteromorphism in *Packera*. Other species in the genus may display germination-based heteromorphism between central and peripheral seeds, including 12 considered threatened or endangered in North America (USDA & NRCS 2012). The detection of cryptic seed heteromorphism in *P. tomentosa*, a species producing central and peripheral seeds without obvious morphological differentiation, supports previous suggestions that cryptic seed heteromorphism is more common than currently documented, particularly in the Asteraceae.

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