



1-2004


# Seed Mass and Morphology in Outcrossing and Selfing Species of *Clarkia* (Onagraceae): An SEM Study

Jennifer L. Knies  
*University of North Carolina at Chapel Hill*

Veronique A. Delesalle  
*Gettysburg College*

A. R. Cavaliere  
*Gettysburg College*

Follow this and additional works at: <https://cupola.gettysburg.edu/biofac>

 Part of the [Biology Commons](#), and the [Plant Biology Commons](#)

**Share feedback about the accessibility of this item.**

---

Knies, Jennifer L., Veronique A. Delesalle and A.R. Cavaliere. Seed Mass and Morphology in Outcrossing and Selfing Species of *Clarkia* (Onagraceae): An SEM Study. *International Journal of Plant Sciences*. (January 2004) 165(1):85-96.

This is the publisher's version of the work. This publication appears in Gettysburg College's institutional repository by permission of the copyright owner for personal use, not for redistribution. Cupola permanent link: <https://cupola.gettysburg.edu/biofac/11>

This open access article is brought to you by The Cupola: Scholarship at Gettysburg College. It has been accepted for inclusion by an authorized administrator of The Cupola. For more information, please contact [cupola@gettysburg.edu](mailto:cupola@gettysburg.edu).

---

# Seed Mass and Morphology in Outcrossing and Selfing Species of *Clarkia* (Onagraceae): An SEM Study

## Abstract

Seeds from three pairs of outcrossing-selfing sister taxa from the genus *Clarkia* (farewell-to-spring, Onagraceae)—*Clarkia unguiculata*, *Clarkia exilis*, *Clarkia xantiana* ssp. *xantiana* and ssp. *parviflora*, and *Clarkia concinna* ssp. *concinna* and ssp. *automixa*—were studied to assess the effects of contrasting mating systems on seed mass and seed morphology. For each outcrossing-selfing comparison, the seed mass of the selfing taxon was less than that of the outcrossing taxon. Seed mass typically differed significantly among populations within a taxon. Scanning electron microscopy showed that the seeds from all these taxa share several characteristics: a bullet to shield shape, a reticulate exotesta pattern, presence of crystals in the seed coat, and a seed coat that varies in thickness over the length of the seed. No morphological feature reliably distinguished seeds of outcrossing taxa from those of selfing taxa. The lack of morphological differences in conjunction with the consistent differences in seed mass between selfing and outcrossing seeds in these taxa supports the hypothesis that evolutionary forces have acted only on seed mass and not on seed morphology.

## Keywords

seed mass, seed morphology, outcrossing, selfing, *Clarkia*, Onagraceae

## Disciplines

Biology | Plant Biology | Plant Sciences

## SEED MASS AND MORPHOLOGY IN OUTCROSSING AND SELFING SPECIES OF *CLARKIA* (ONAGRACEAE): AN SEM STUDY

Jennifer L. Knies,<sup>1,\*</sup> Véronique A. Delesalle,<sup>†</sup> and A. R. Cavaliere<sup>†</sup>

\*Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A.; and

<sup>†</sup>Department of Biology, Gettysburg College, Gettysburg, Pennsylvania 17325, U.S.A.

Seeds from three pairs of outcrossing-selfing sister taxa from the genus *Clarkia* (farewell-to-spring, Onagraceae)—*Clarkia unguiculata*, *Clarkia exilis*, *Clarkia xantiana* ssp. *xantiana* and ssp. *parviflora*, and *Clarkia concinna* ssp. *concinna* and ssp. *automixa*—were studied to assess the effects of contrasting mating systems on seed mass and seed morphology. For each outcrossing-selfing comparison, the seed mass of the selfing taxon was less than that of the outcrossing taxon. Seed mass typically differed significantly among populations within a taxon. Scanning electron microscopy showed that the seeds from all these taxa share several characteristics: a bullet to shield shape, a reticulate exotesta pattern, presence of crystals in the seed coat, and a seed coat that varies in thickness over the length of the seed. No morphological feature reliably distinguished seeds of outcrossing taxa from those of selfing taxa. The lack of morphological differences in conjunction with the consistent differences in seed mass between selfing and outcrossing seeds in these taxa supports the hypothesis that evolutionary forces have acted only on seed mass and not on seed morphology.

*Keywords:* seed mass, seed morphology, outcrossing, selfing, *Clarkia*, Onagraceae.

### Introduction

Seed coat morphology and surface sculpturing are generally useful traits for species identification (Chance and Bacon 1984; Matthews and Levine 1986; Shetler and Morin 1986) and taxonomy (Seavey et al. 1977; Bobrov et al. 1999). Seed morphology, alone or with DNA analysis, has been used to reconstruct phylogeny at the genus and species levels (Chuang and Ornduff 1992; Hufford 1995; Kumar et al. 1999). In the genus *Ludwigia* (Onagraceae), variation in seed coat anatomy and specialization of the seed coat layers make the seed coat one of the most useful features to determine relationships between living and extinct taxa (Tobe and Raven 1986; Tobe et al. 1988). In the genus *Oenothera* (Onagraceae), seed coat anatomy has been used to restructure sections and determine evolutionary relationships among sections (Tobe et al. 1987).

Beginning with the work of Lewis and Lewis (1955), the genus *Clarkia* (Onagraceae) has been extensively used in studies of plant breeding system evolution (Lewis and Raven 1958; Bartholemew et al. 1973; Gottlieb 1984; Vasek and Weng 1988; Holtsford and Ellstrand 1992). Within this genus, selfing has evolved independently at least 12 times (Lewis and Raven 1958; Runions and Geber 2000), with selfing taxa (whether species or subspecies) typically distributed parapatrically to and occupying more marginal (typically drier) habitats than those of their sister outcrossing taxa (Lewis and Lewis 1955). For example, the selfing *Clarkia*

*xantiana* ssp. *parviflora* is found to the eastern edge of and in drier habitats than its outcrossing subspecies *xantiana* (Eckhart and Geber 1999). Selfing taxa typically have smaller plants with smaller flowers, lower pollen : ovule (P : O) ratios, and little herkogamy or protandry (Vasek and Weng 1988; Eckhart and Geber 1999; Runions and Geber 2000).

In this study, we wanted to determine whether the ecological differences in the habitats of selfers compared with outcrossers were coupled with differences in their seed characteristics. Differences in habitat characteristics have been linked to differences in germination behavior in a number of related species (Araki and Washitani 2000; Kamenetsky and Gutterman 2000; Andersson et al. 2002). For example, in the genus *Bromus*, *Bromus sterilis* and *Bromus tectorum* exhibit stronger dormancy than related species *Bromus avensis* and *Bromus hordeaceus*. *Bromus sterilis* and *B. tectorum* inhabit agricultural fields, and this strong dormancy allows their seeds to remain ungerminated until crops have been sowed (Andersson et al. 2002). Germination is regulated by the seed coat, which protects, feeds, and regulates the water intake of seeds (Murray 1984); thus, adaptive differences in germination cues to water could be reflected in seed morphology. Specifically, we expected that, compared with their outcrossing sister taxa, the seeds of the selfing taxa would exhibit differences in seed mass, shape, and coat thickness that would only allow germination under conditions highly favorable for growth (e.g., heavy rainfall).

No research has been done on the seed morphology of the genus *Clarkia*. Moreover, we know of no research comparing the seed morphology of related species with contrasting mating systems. Consequently, the genus *Clarkia* is ideal for this study. Here we determine the evolutionary effects of breeding system on seed mass and morphology of three

<sup>1</sup> Author for correspondence; e-mail knies@email.unc.edu.

**Table 1**  
**List of *Clarkia* Populations Sampled**

Taxon and population	Location	Population size
<i>Clarkia exilis</i> :		
Deer Creek Rd. (DC) <sup>a</sup>	Tulare Co. (35°55.18'N, 118°47.45'W)	Medium-large
Upper Rich Bar (URB)	Kern Co. (Hwy. 178, 9.3 mile NE from Rancheria Rd.)	Small-medium
Kern Canyon Rd. (KCR)	Kern Co. (Kern Canyon Rd. at mile post 27.19)	Small-medium
Cow Flat Creek (CF) <sup>a</sup>	Kern Co. (35°29.96'N, 118°41.65'W)	Medium
Granite Station (GS) <sup>a</sup>	Kern Co. (35°37.06'N, 118°51.50'W)	Small
<i>Clarkia unguiculata</i> :		
Jolon Rd. (JR) <sup>a</sup>	Monterey Co. (from Rte. 101, 10 mi NW on Jolon Rd.)	Medium-large
Upper Rich Bar (URB)	Kern Co. (see above)	Medium-large
Kern Canyon Rd. (KCR)	Kern Co. (see above)	Medium
Wofford Heights (WH) <sup>a</sup>	Kern Co. (35°43.26'N, 118°30.04'W)	Large
<i>Clarkia xantiana</i> ssp. <i>parviflora</i> :		
French Gulch (FG) <sup>a</sup>	Kern Co. (11.0 mi S of Kernville on Hwy. 155)	Medium
Sawmill (SM) <sup>a</sup>	Kern Co. (5.5 mi S of Kernville on Hwy. 155)	Medium
Long Valley (LV)	Kern Co. (35°48.88'N, 119°05.65'W)	Large
<i>C. xantiana</i> ssp. <i>xantiana</i> :		
Wofford Heights (WH) <sup>a</sup>	Kern Co. (35°43.26'N, 118°30.04'W)	Large
South Creek (SC) <sup>a</sup>	Tulare Co. (Mtn. 99 at 2.00-mile marker W of Johnsondale Bridge)	Small
<i>Clarkia concinna</i> ssp. <i>automixa</i> :		
Mt. Hamilton Rd. (MH) <sup>a</sup>	Santa Clara Co. (Joseph Grant County Park)	Small
<i>C. concinna</i> ssp. <i>concinna</i> :		
Rte. 175 (Rte. 175) <sup>*</sup>	Lake Co. (38°58.33'N, 123°01.37'W)	Medium-large
Alder Glen (AG)	Sonoma Co. (38°50.62'N, 123°04.03'W)	Medium-large
Willits (W) <sup>a</sup>	Mendocino Co. (39°23.33'N, 123°25.89'W)	Medium

Note. Populations were classified as small (<50 individuals), medium (50–500 individuals), or large (>500 individuals) by V. A. Delesalle on the basis of field observations. For locations lacking GPS data, exact locations can be obtained from V. A. Delesalle. Seeds from all populations, except GS and FG, were weighed for seed mass data.

<sup>a</sup> Population used in the seed coat morphology study.

outcrossing-selfing sister taxon pairs: *Clarkia unguiculata* and *Clarkia exilis* (section Phaeostoma), *C. xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora* (section Phaeostoma), and *Clarkia concinna* ssp. *concinna* and *C. concinna* ssp. *automixa* (section Eucharidium). These species were chosen because they are all diploid, the sister taxon relationships are well supported (Systma and Smith 1988), and selfers and outcrossers are found in different habitats (Vasek and Harding 1976; Allen et al. 1991; Eckhart and Geber 1999).

## Material and Methods

### Species Description

The Onagraceae consist of seven tribes, 17 genera, and 674 species (Tobe and Raven 1986). Within this family, the annual genus *Clarkia* has been widely studied. This genus numbers 43 species inhabiting a wide range of habitats in western North America (Lewis and Lewis 1955), with one species native to South America (Raven 1988). Seeds typically germinate after the winter rains from December to February. Plants flower late in the spring (April–June), and fruits dehisce and seeds disperse ca. 1 mo after fruit set (V. A. Delesalle, personal observation). All species are self-compatible, with outcrossers typically exhibiting strong dichogamy and herkogamy (Holtsford and Ellstrand 1992; V. A. Delesalle, personal observation). Selfers typically flower earlier than outcrossers and produce smaller flowers with lower P : O ra-

tio than outcrossers (Vasek and Weng 1988; Eckhart and Geber 1999; Runions and Geber 2000; V. A. Delesalle, personal observation). In the species studied, the selfing taxon inhabits a smaller and more marginal range compared with the outcrossing taxon. Each pair differs in the degree of outcrossing: outcrossers are best described as predominantly outcrossing and are capable of self-fertilization, while selfers are predominantly selfing but capable of xenogamy. In the greenhouse, selfers flower earlier than outcrossers (seeds started at the same time) and exhibit high levels of unaided self-pollination; in contrast, outcrossers set few fruits in an insect-free greenhouse (V. A. Delesalle, personal observation).

In the current taxonomy of the genus, *Clarkia unguiculata* and *Clarkia xantiana* are held to be more closely related to one another than either species is to *Clarkia concinna* (Lewis and Lewis 1955; Systma and Smith 1988). *Clarkia unguiculata* and *C. xantiana* are hypothesized to split at a polychotomous node or align with species in other sections of the genus. In contrast, the *C. concinna* taxon is hypothesized to be either in the same lineage as *C. unguiculata* and *C. xantiana* or removed from their sections and in a separate lineage (Systma and Smith 1988).

### *Clarkia unguiculata* and *Clarkia exilis*

Most probably derived from the outcrossing *C. unguiculata*, *C. exilis* is self-pollinating and endemic to the Kern and Tulare counties of California, while *C. unguiculata* is

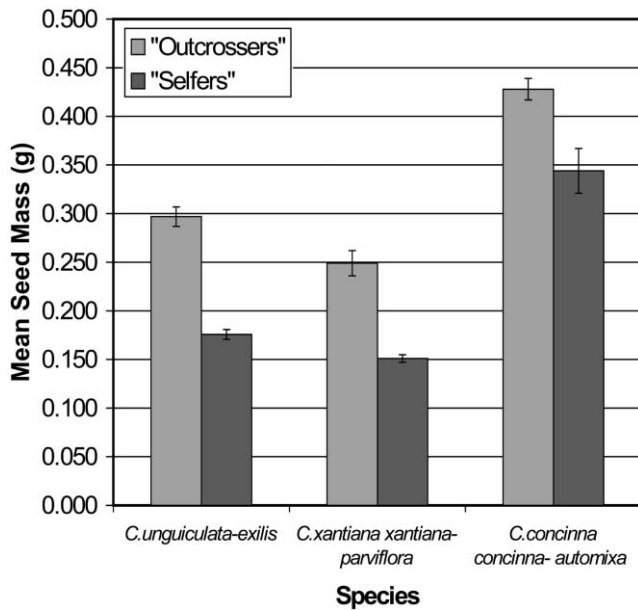


Fig. 1 Mean seed mass for each sister taxon. Data for each population (from table 2) were combined to determine mean seed mass for each taxon. Means  $\pm$  1 SE are shown.

widely distributed throughout California (Lewis and Lewis 1955). In the field, *C. exilis* flowers and fruits before *C. unguiculata* and is restricted to wetter habitats (V. A. Delesalle, personal observation). *Clarkia unguiculata* is highly outcrossing (95%–100%), while *C. exilis* populations have a lower outcrossing rate (38%, 44%) (Vasek and Harding 1976). Associated with these mating system differences, *C. unguiculata* produces fewer ovules per ovary (93.1) and has a higher P : O ratio (158.5 : 1) than *C. exilis* (115.4 ovules per ovary; P : O ratio, 52.5 : 1) (Vasek and Weng 1988).

*Clarkia xantiana ssp. xantiana and*  
*Clarkia xantiana ssp. parviflora*

Outcrossing *C. xantiana ssp. xantiana* and selfing *ssp. parviflora* inhabit the southern Sierras of California (Lewis and Lewis 1955). *Clarkia xantiana ssp. parviflora* inhabits more xeric habitats than *C. xantiana ssp. xantiana* (Eckhart and Geber 1999). In sympatric populations, selfers flower before outcrossers. Similar to the *C. unguiculata/exilis* pair, the selfer has a higher mean ovule number (115.7) than the outcrosser (90.2) and a lower mean P : O ratio (36.1 : 1 vs. 136.6 : 1) (Vasek and Weng 1988).

*Clarkia concinna ssp. concinna and*  
*Clarkia concinna ssp. automixa*

*Clarkia concinna ssp. concinna* and *C. concinna ssp. automixa* are morphologically distinct from the previous species given their shorter size, more profuse branching, and production of fewer flowers. Mating system differences between the subspecies have been inferred on the basis of their flower morphology (Bowman 1987) and electrophoresis (Allen et al. 1991). Outcrossing *C. concinna ssp. concinna* inhabits the

northern coast of California and extends into central California with increasing latitude. In contrast, the selfing *C. concinna ssp. automixa* inhabits a small range on the southern end of the outcrosser's range in Santa Clara and Alameda counties (Lewis and Lewis 1955). There is no published information on the P : O ratio in this species. V. A. Delesalle (unpublished data) found that *C. concinna ssp. concinna* flowers produce 21–92 ovules (mean = 38.2) and 1210–6030 pollen grains (mean = 3795) for a P : O ratio of 99.3 : 1.

*Seed Data*

In the summers of 1999 and 2001, we collected seeds from a number of populations for all the above taxa. The sampled populations were diverse in size and location (table 1, list of population abbreviations). Seeds were stored dry at 5°C. For each population, mean seed mass was obtained by weighing on a microbalance one seed per maternal plant for up to 30 maternal families per population. To estimate mean seed mass for each taxon, population data were combined. Seed length, width, and coloring were determined under light microscopy. Seed length and width were measured to the nearest 1/100 mm at the seed's longest and widest points, respectively, for one seed per maternal plant for five maternal families from each sampled population.

*Statistical Analyses*

For each outcrossing-selfing pair, we tested the hypothesis that the seed mass of the outcrossing taxon differed from

Table 2

Mean Seed Mass for Each Sampled Population		
Taxon and population	Mean seed mass $\pm$ 1 SE (mg)	<i>n</i>
<i>Clarkia exilis</i> :		
KCR	0.163 $\pm$ 0.009 <sup>A</sup>	21
URB	0.167 $\pm$ 0.012 <sup>A</sup>	11
CF	0.188 $\pm$ 0.006 <sup>B</sup>	30
<i>Clarkia unguiculata</i> :		
JR	0.251 $\pm$ 0.016 <sup>C</sup>	30
KCR	0.272 $\pm$ 0.019 <sup>C</sup>	17
WH	0.328 $\pm$ 0.019 <sup>D</sup>	30
URB	0.347 $\pm$ 0.014 <sup>D</sup>	17
<i>Clarkia xantiana ssp. parviflora</i> :		
SM	0.140 $\pm$ 0.003 <sup>A</sup>	30
LV	0.163 $\pm$ 0.007 <sup>AB</sup>	30
<i>C. xantiana ssp. xantiana</i> :		
WH	0.207 $\pm$ 0.008 <sup>B</sup>	30
SC	0.292 $\pm$ 0.023 <sup>C</sup>	29
<i>Clarkia concinna ssp. automixa</i> :		
MH	0.344 $\pm$ 0.023 <sup>A</sup>	12
<i>C. concinna ssp. concinna</i> :		
Rte. 175	0.360 $\pm$ 0.013 <sup>A</sup>	30
AG	0.434 $\pm$ 0.015 <sup>B</sup>	27
W	0.501 $\pm$ 0.017 <sup>C</sup>	25

Note. For each population, one seed per family up to 30 families were weighed. For each pair of selfing-outcrossing sister taxa, means followed by different letters were statistically different on the basis of Bonferroni/Dunn post hoc tests. *n* = the number of families sampled per taxon.

**Table 3**  
**Mean Seed Mass, Seed Width, and Seed Length for Each Taxon**

Taxon	Mean seed mass (mg)	Mean seed width (mm)	Mean seed length (mm)	<i>n</i>
<i>Clarkia exilis</i>	0.176	0.66	1.01	62 (15)
<i>Clarkia unguiculata</i>	0.297	0.84	1.27	94 (20)
<i>Clarkia xantiana</i> ssp. <i>parflivora</i>	0.151	0.67	1.20	60 (10)
<i>C. xantiana</i> ssp. <i>xantiana</i>	0.249	0.71	1.29	59 (10)
<i>Clarkia concinna</i> ssp. <i>automixa</i>	0.344	0.74	1.98	12 (5)
<i>C. concinna</i> ssp. <i>concinna</i>	0.428	0.91	2.32	82 (15)

Note. Population data were combined for taxon means. Mean seed length and width were determined by measurements made at the seeds' longest and widest points, respectively, under light microscopy. *n* = the number of maternal families sampled for mean seed mass data. Counts in parentheses indicate sample size for the seed length and width measurements.

that of its related selfing taxon. We sampled too few populations to nest populations within taxon in our analyses. As a consequence, we performed one-way ANOVAs comparing mean seed mass among populations and used post hoc tests (Bonferroni/Dunn) to determine whether populations from the outcrossing taxon differed from populations of the selfing taxon. Analyses were conducted separately for each outcrossing-selfing pair.

#### Study of Seed Morphology

External and cross-sectional seed morphology were studied under scanning electron microscopy (SEM). Seed shape, exotesta cell shape, and patterns were documented for five to 10 seeds per population (and for five to 20 seeds per taxon). In addition, number, thickness, and morphology of the coat layers were examined by making longitudinal and lateral cross-sections of the seeds. Razor blade sectioning can be and was used successfully to study the fine features of the seed coat (Chance and Bacon 1984). Damage to cross-sectioned seeds was easily detected under SEM, and damaged seeds were discarded.

Cross-sectioned seeds were fixed in Karnovsky's fixative for 2–5 hr, washed in three changes of cacodylate buffer, and dehydrated in increasing alcohol concentrations. These seeds then underwent critical point drying with a Tousimis Samdri-790 critical point dryer. Cross-sectioned and whole seeds were mounted on metal stubs using double stick tape, gold coated to ca. 10–20 nm, then examined with a JEOL 5200 SEM at 15 kV. Seed surface ultrastructure and cross-sectional morphology were studied at different magnifications, and images were either photographed or digitally stored.

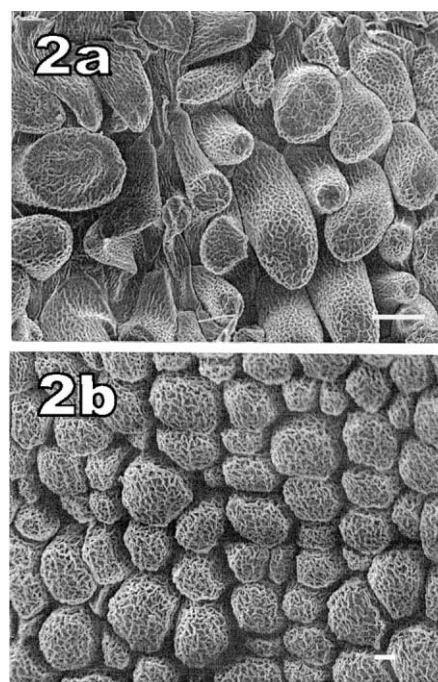
## Results

### Seed Mass

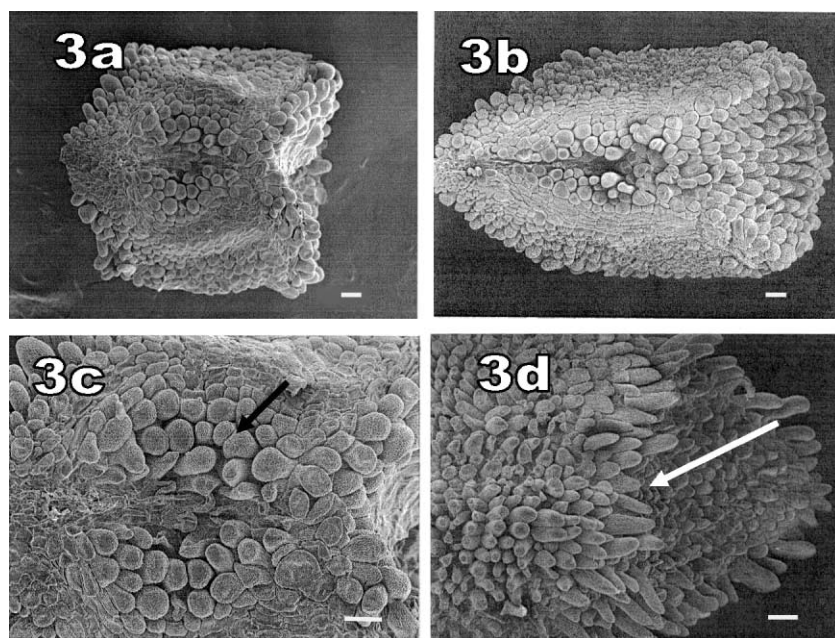
In all samples, the outcrossing taxon's mean seed mass was greater than that of its selfing sister taxon (fig. 1). Moreover, greater seed mass was associated with longer and wider seeds (tables 2, 3). The mean population seed mass for *Clarkia unguiculata* ranged from 0.251 to 0.347 mg and was nearly twice the mean population seed mass of *Clarkia exilis* (table

2; 0.163–0.188 mg; population effect,  $F = 22.496$ ,  $df = 6, 149$ ,  $P < 0.0001$ ). On the basis of Bonferroni/Dunn post hoc tests, all *C. unguiculata* populations had larger mean seed mass than all *C. exilis* populations. Within *C. unguiculata*, the Upper Rich Bar (URB) and Wofford Heights (WH) populations had larger mean seed mass than the Jolon Road (JR) and Kern Canyon Road (KCR) populations. Within *C. exilis*, the Cow Flat Creek (CF) population had a larger mean seed mass than the KCR and URB populations, which did not differ significantly from one another.

For the two *Clarkia xantiana* subspecies, we detected variation among populations in mean seed mass (table 2;  $F = 28.779$ ,  $df = 3, 115$ ,  $P < 0.0001$ ). On the basis of Bon-



**Fig. 2** Common features of *Clarkia* seeds. *a*, Reticulate pattern covering exotesta. *b*, Compact exotesta cells on the nonhilum side of the seed. Scale bars = 50  $\mu$ m (*a*) and 10  $\mu$ m (*b*).

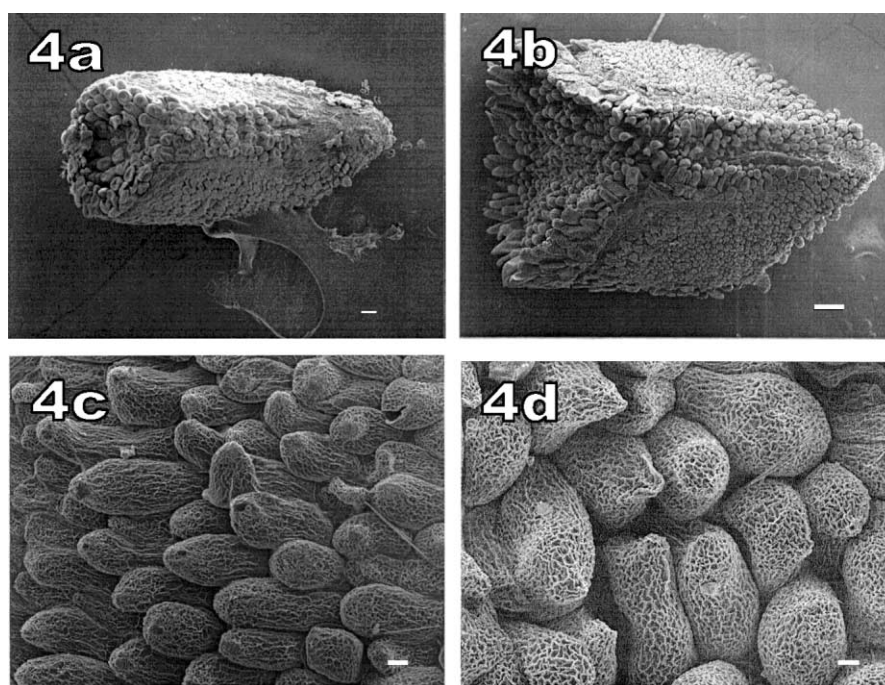


**Fig. 3** External morphology of *Clarkia unguiculata*. *a*, Shield-shaped Wofford Heights seed. *b*, Bullet-shaped Jolon Road seed. *c*, Exotesta cells circling the hilum. *d*, “Fringe” of exotesta cells surrounding the tapered end (at arrow). Scale bars = 0.100 cm.

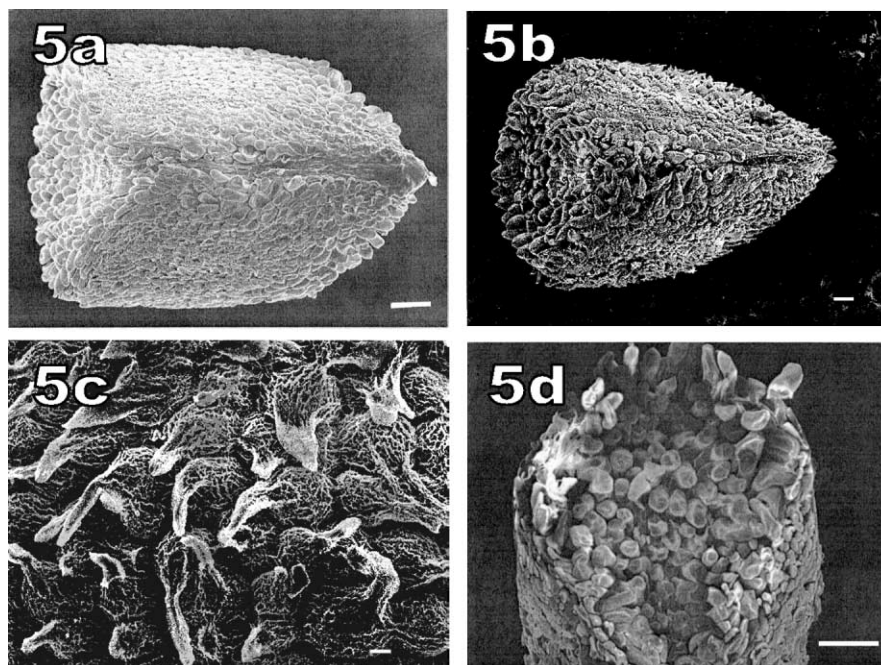
ferroni/Dunn post hoc tests, the outcrossing South Creek (SC) population (0.292 mg) had a larger mean seed mass than all other populations. The outcrossing WH population (0.207 mg) and the selfing Long Valley (LV) population (0.163 mg) did not differ significantly in seed mass, but WH

plants produced larger seeds than plants of the selfing Sawmill (SM) population (0.140 mg), and the two selfing populations did not differ significantly from one another.

We also detected variation among populations in mean seed mass for the two *Clarkia concinna* subspecies, (table 2;



**Fig. 4** External morphology of *Clarkia exilis*. *a*, Deer Creek seed. *b*, Granite Station seed. *c*, Elongated exotesta cells surrounding the hilum. *d*, Irregularly shaped exotesta cells at the truncated end. Scale bars = 0.100 cm (*a*, *b*) and 10  $\mu$ m (*c*, *d*).



**Fig. 5** External morphology of *Clarkia xantiana* ssp. *xantiana*. *a*, South Creek seed. *b*, Wofford Heights seed. *c*, Gour-shaped Wofford Heights exotesta cells. *d*, Rounded South Creek exotesta cells. Scale bars = 0.100 cm (*a*, *b*, *d*) and 10  $\mu$ m (*c*).

$F = 18.600$ ,  $df = 3, 90$ ,  $P < 0.0001$ ). On the basis of Bonferoni/Dunn post hoc tests, the outcrossing Willits (W) population (0.501 mg) had a larger mean seed mass than all other populations. The outcrossing Alder Glen (AG) population (0.434 mg) produced larger seeds than the outcrossing Route 175 population (0.360 mg) and the selfing Mount Hamilton (MH) population (0.344 mg), which did not differ significantly from one another.

#### External Seed Morphology: Common Morphological Features

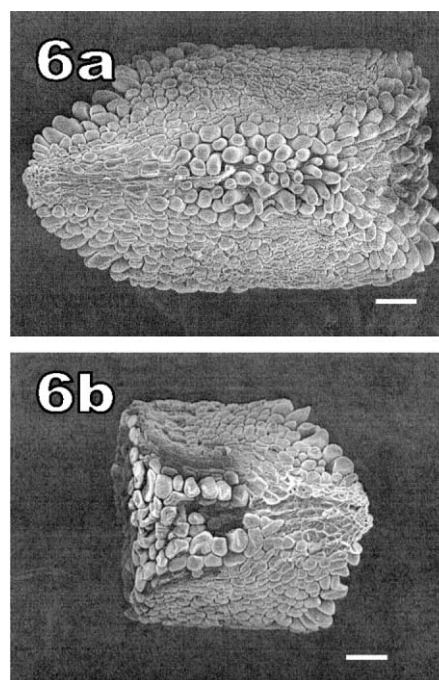
Seeds from all taxa share several characteristics. The seeds are small, averaging between 0.66–0.91 mm in width and 1.01–2.32 mm in length (table 3). Seeds of *C. concinna* are heavier and longer, but not wider, than seeds of the other species. Seeds are brown to black in color, have a common shape (bullet or shield shape) with a truncated and a tapered end, and are covered by a reticulate patterned exotesta (fig. 2*a*). In nearly all populations, the exotesta cells on the hilum side of the seed are loose and more elongated than the compact exotesta cells on the remainder of the seed (fig. 2).

#### Variable Features

***Clarkia unguiculata* and *Clarkia exilis*.** The two *C. unguiculata* populations differ in their seed shape. WH seeds are shield shaped, while JR seeds are bullet shaped (fig. 3*a*, 3*b*). The WH exotesta cells surround the hilum circumferentially (fig. 3*c*), and the JR exotesta cells surround the hilum in a nearly linear fashion (fig. 3*b*). In seeds from both populations, several rows of longitudinally flat exotesta cells surround the radially elongated exotesta cells around the hilum.

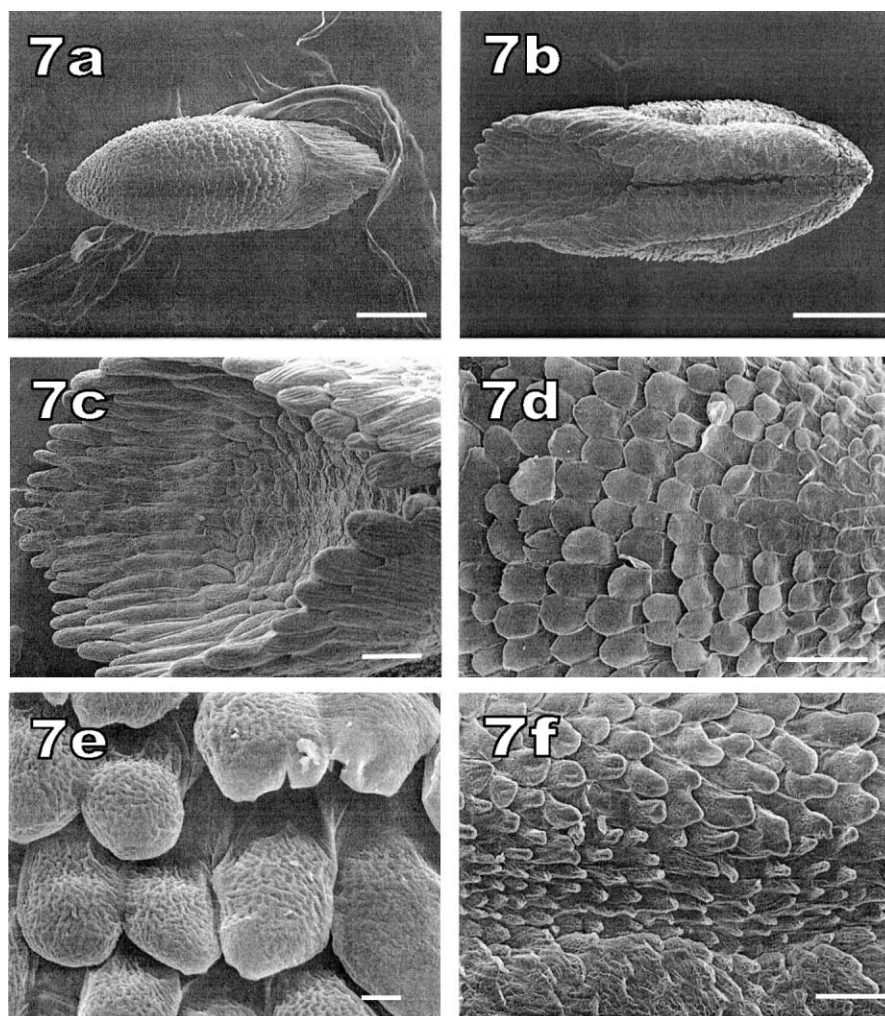
In addition, a “fringe” of extraradially elongated exotesta cells surrounds the tapered end (fig. 3*d*).

The three *C. exilis* populations have a common bullet-shaped seed (fig. 4*a*, 4*b*). Five to 11 rows of collapsed



**Fig. 6** External morphology of *Clarkia xantiana* ssp. *parviflora*. *a*, French Gulch seed. *b*, Sawmill seed. Scale bars = 0.100 cm.





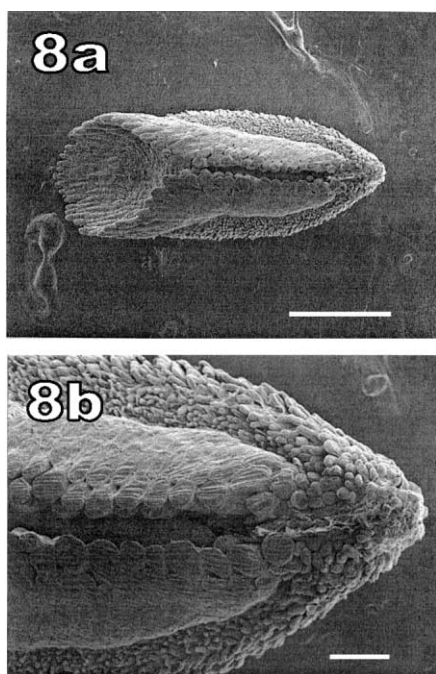
**Fig. 7** External morphology of *Clarkia concinna* ssp. *concinna*. *a*, Nonhilum side of seed. *b*, Hilum side of seed. *c*, Truncated end of the seed. *d*, Ridged exotesta cells on the nonhilum side of the seed. *e*, At a higher magnification, reticulate pattern becomes apparent on the ridged exotesta cells. *f*, Exotesta cells change shape from the nonhilum to the hilum side. Scale bars = 0.500 cm (*a*, *b*), 0.100 cm (*c*, *d*), 10  $\mu$ m (*e*), and 50  $\mu$ m (*f*).

exotesta cells surround the elongated cells (fig. 4c) around the hilum side. The truncated exotesta cells are irregular in shape (fig. 4d), ranging from clavate to subspherical or spherical. The Granite Station (GS) population differs from the other two populations of *C. exilis* in several features. Their most elongated exotesta cells extend to the tapered end of the seed. A “fringe” of loose extraradially elongated cells surrounds the truncated and tapered ends (fig. 4d).

***Clarkia xantiana* ssp. *xantiana* and *Clarkia xantiana* ssp. *parviflora*.** Seeds from both *C. xantiana* ssp. *xantiana* populations are bullet shaped (fig. 5a, 5b) but differ in the shape of their exotesta cells. The WH cells are gourd shaped, with a wide base that quickly tapers to a narrow elongated tip (fig. 5c), while the SC cells are of even width and more spherical (fig. 5d). A “fringe” of extraelongated loose exotesta cells surrounds the truncated (chalazal) end. The largest exotesta cells are continuous with the chalazal exotesta ring and arranged linearly from the chalazal to the tapered seed end.

The *C. xantiana* ssp. *parviflora* populations exhibit bullet-shaped and shield-shaped seeds. French Gulch (FG) seeds are strictly bullet shaped, while SM seeds range from shield to bullet shaped (fig. 6). Rounded exotesta cells characterize both populations.

Seeds of *C. xantiana* ssp. *xantiana* and ssp. *parviflora* are indistinguishable from those of *C. unguiculata* and *C. exilis*. Considerable overlap in seed morphology exists between the species described so far. Several populations show unique seed characteristics (seed shape, exotesta shape). The *C. exilis* GS seeds are morphologically distinct from all other populations principally because of the extraradially elongated cells that surround the truncated seed end. The *C. xantiana* ssp. *xantiana* WH seeds can be distinguished from all other populations by the shape of their exotesta cells. However, no morphological trait differentiates selfing and outcrossing taxa (*C. unguiculata* vs. *C. exilis* and *C. xantiana* ssp. *xantiana* vs. ssp. *parviflora*).



**Fig. 8** External morphology of *Clarkia concinna* ssp. *automixa*. *a*, Hilum side of seed. *b*, Close-up of hilum (recurved exotesta over protruding cells). Scale bars = 0.500 cm (*a*) and 0.100 cm (*b*).

***Clarkia concinna* ssp. *concinna* and *Clarkia concinna* ssp. *automixa*.** The *C. concinna* ssp. *concinna* populations have distinct nonhilum and hilum sides (fig. 7*a*, 7*b*). The convoluted recurving of the exotesta over the hilum side and the rocket-shaped seed distinguishes *C. concinna* ssp. *concinna* and ssp. *automixa* from the previous two pairs of taxa. The recurving of the exotesta causes partial or complete covering of two-thirds of the hilum-side exotesta. In both populations, exotesta cells on the hilum side are collapsed except for the chalazal cells and the recurved exotesta cells. The chalazal end is deeply concave and ringed by flattened, elongated exotesta cells (fig. 7*c*). The recurved exotesta cells are longitudinally elongated and patterned with transverse striations. The nonhilum side of the seed is covered by lateral rows of fused, ridged exotesta cells (fig. 7*d*). These exotesta cells are covered with a veined pattern apparent at high magnifications (fig. 7*e*). Moving from the nonhilum to hilum seed side, the exotesta cells are more radially elongated, are no longer ridged, and decrease in width (fig. 7*f*).

The *C. concinna* ssp. *automixa* seeds are also rocket shaped (fig. 8*a*), with a distinct hilum and nonhilum side. At the narrower end of the seed, the exotesta recurves above itself, and this recurving covers the hilum. Occasionally, the exotesta layer protrudes between the recurved exotesta cells on the hilum side (fig. 8*b*). This was observed only in *C. concinna* ssp. *automixa*. Otherwise, the external seed morphology of *C. concinna* ssp. *automixa* is apparently the same as that of *C. concinna* ssp. *concinna*.

In summary, the seed morphology of all three taxon pairs shows considerable overlap, and no unique features distinguish the selfing taxon from its outcrossing sister taxon.

### Internal Seed Morphology

As is typical of the Onagraceae, none of the species surveyed have endosperm within their seeds. The two cotyledons are visible and fill the entire seed. Crystals (CR) are prevalent along the hilum side of the seed, particularly near the hilum itself and at the truncated end of the seed. For all taxa, the crystals are in the collapsed exotesta cells (fig. 9*a*).

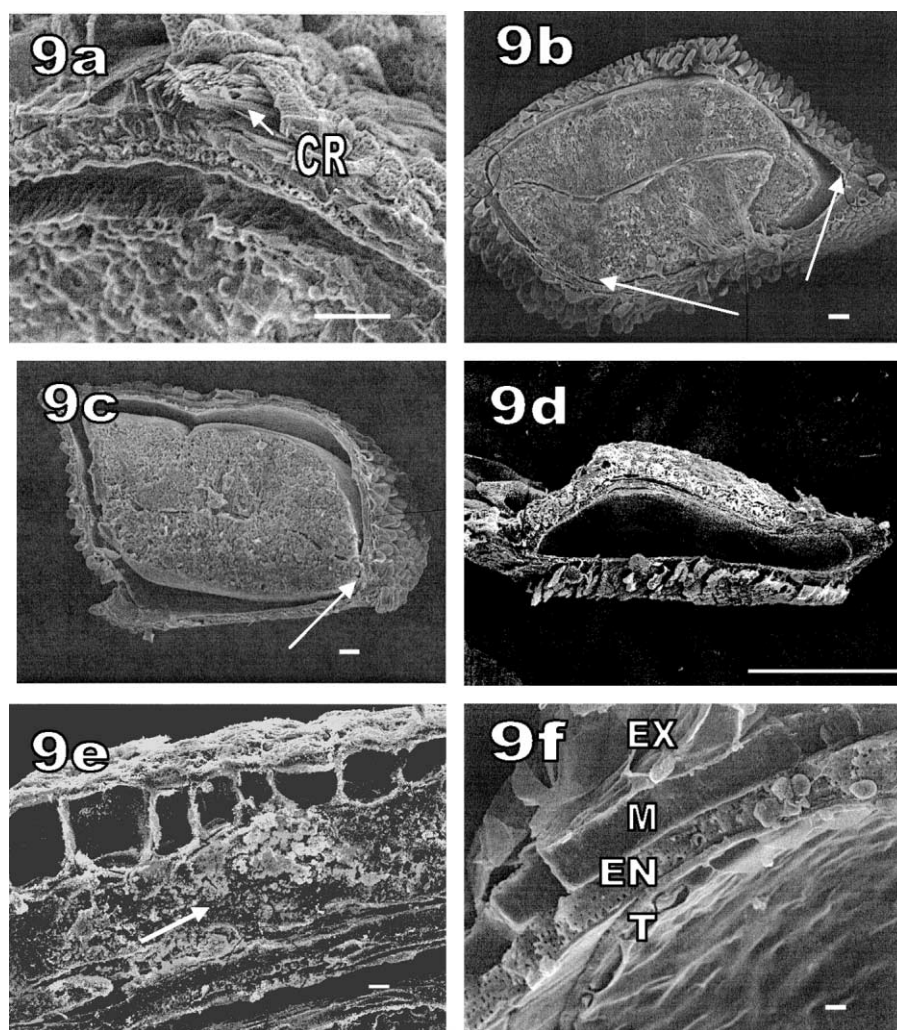
For all the seeds examined, the coat layers vary in thickness along the length of the seed (fig. 9*b*–9*d*; table 4). Variation in thickness was consistent regardless of the angle of cross-sectioning. The seed coat of *C. concinna* and its subspecies *automixa* have a curious appearance on the hilum side, where an apparent additional layer sits in the center of the seed coat. This layer is thick and not similar in appearance to any other layer (fig. 9*e*).

For all taxa, the seed coat is thickest (see high-end values in table 4) at the tapered end of the seed coat and at the “corner” of the truncated end (arrows in fig. 9*b*, 9*c*). The remainder of the seed coat is much thinner than these regions (see low-end values in table 4). Crystals (CR) occur at the hilum and truncated end of the seed. A tegmen (T), endotesta (EN), mesotesta (M), and exotesta (EX) are found throughout the seed coat (fig. 9*f*). The tegmen cells are typically indistinguishable and compressed except toward the hilum, where they become much larger and thinner walled. The endotesta cells are pitted and thick walled. The mesotesta and exotesta cells are thin walled. The tegmen is always the thinnest of the layers, while the endotesta and mesotesta can be about equal in thickness or one layer can be disparately larger than the other (table 3).

## Discussion

### Seed Mass

In all sister taxa comparisons, the seeds of the selfing taxon were smaller than those of the outcrossing taxon (tables 2, 3; fig. 1). The significant difference in seed mass between species with contrasting mating systems indicates different adaptive strategies may be at work. Smaller seeds are typical for species living in ephemeral or noncompetitive habitats (Salisbury 1942; Baker 1972; Grime 1977; Mazer 1989; Leishman et al. 1995; Thompson and Hodkinson 1998). In addition, selfing taxa produce more ovules per ovary and presumably have more seeds per fruit than outcrossing taxa (V. A. Delesalle and S. Mazer, unpublished data). Under conditions of high mortality, an increased number of smaller seeds may increase the dispersal range of seeds and/or the likelihood that some seeds will survive to the seedling stage. Given the trade-off between seed dispersal and size (Smith and Fretwell 1974; Venable and Brown 1988) and the passive dispersal mode of *Clarkia* seeds, we hypothesize that the smaller seeds of the selfers should disperse farther than the larger seeds of the outcrossers and, consequently, increase their likelihood of landing in an environment favorable for germination. In contrast, the greater mass of outcrossed seeds should increase their seeds’ likelihood of germination and survival under stressful/harsher conditions.



**Fig. 9** Features of cross-sectioned *Clarkia* seeds. *a*, Crystals (CR) apparent in the exotesta cells. *b*, *c*, Arrows denote the thickest areas of the seed coat. *b*, Example of *Clarkia unguiculata* longitudinal cross-sectioned (LC) seed. *c*, Example of *Clarkia xantiana* ssp. *xantiana* LC seed. *d*, Example of *Clarkia concinna* ssp. *concinna* LC seed. *e*, Seed coat of *C. concinna* ssp. *concinna* with an arrow showing the undefined layer found on the hilum side. *f*, Layers of the seed coat: exotesta (EX), mesotesta (M), endotesta (EN), and tegmen (T). Scale bars = 50  $\mu\text{m}$  (*a*), 0.100 cm (*b*, *c*), 0.500 cm (*d*), and 10  $\mu\text{m}$  (*e*, *f*).

A smaller seed size also facilitates seed integration into the soil, where seeds live longer (Venable and Brown 1988). Small seed size is correlated with a persistent seed bank (Thompson et al. 1993), which provides a reservoir of genetic diversity (Tonsor et al. 1993; Bekker et al. 1998). For the selfing species of this study, a belowground seed bank might provide a reservoir of genetic diversity, as it does for the related selfing *Clarkia springvillensis*, which has a greater genetic diversity in its seed bank than in its germinated plants (McCue and Holtsford 1998). With longer seed persistence in a seed bank, germination is selected to occur only under the most favorable conditions (Templeton and Levine 1979; Brown and Venable 1986). In contrast, taxa with larger seeds have greater resources for germination (Baker 1972), germinate under a greater range of conditions (Wulff 1986), and would presumably have a smaller seed bank than their relatives with smaller seeds.

We lack information on the seed banks of the studied species. On the basis of our seed mass data, we hypothesize that the selfers have a persistent seed bank, which allows these taxa to germinate only under more favorable conditions than the sampled outcrossers. However, these selfing taxa may produce smaller seeds because they are in nutrient-poor environments and the differences in seed mass we observed may reflect phenotypic plasticity rather than genetic differences. We lack the data to differentiate between these two possibilities.

The seed number-size trade-off seen in our selfing species differs from that of other selfing *Clarkia* (e.g., *Clarkia calientensis* and *Clarkia tembloriensis*; section *Phaeostoma*). These two species produce fewer and larger seeds than their outcrossing counterparts, suggesting adaptations by these selfers to more competitive environments (Vasek and Weng 1988). We need ecological studies to determine the benefits, if any, of these different seed number-size trade-offs.

**Table 4**  
**Seed Coat Thickness and Number of Layers for Seeds of Each Taxon**

Taxon	Tegmen		Endotesta		Mesotesta		Seed coat thickness ( $\mu\text{m}$ )	<i>n</i>
	Thickness ( $\mu\text{m}$ )	Layers	Thickness ( $\mu\text{m}$ )	Layers	Thickness ( $\mu\text{m}$ )	Layers		
<i>Clarkia exilis</i>	1.25–5.39	2	3.63–17.93	1	3.38–17.17	1–2	12.67–41.98	15
<i>Clarkia unguiculata</i>	1.79–5.01	2–4	3.64–26.36	1	4.07–10.38	1–2	11.16–61.24	11
<i>Clarkia xantiana</i> ssp. <i>parflivora</i>	1.28–4.08	1–3	2.36–20.55	1	3.21–7.54	1	6.90–28.04	5
<i>C. xantiana</i> ssp. <i>xantiana</i>	1.83–5.22	1–2+	3.73–10.36	1	4.54–20.76	1	10.66–39.93	5
<i>Clarkia concinna</i> ssp. <i>automixa</i>	2.19–14.59	1–2	3.61–20.95	1	7.49–18.79	1–2	23.30–76.95	7
<i>C. concinna</i> ssp. <i>concinna</i>	2.00–7.47	1	5.60–22.71	1	13.21–20.13	1–2	27.52–49.78	6

Note. Seed coat thickness was measured with the digital scanning generator system. Seeds were cross-sectioned with razor blades, fixed in Karnovsky's fixative, washed in cacodylate buffer, dehydrated in successive levels of acetone (25%–100%), critical point dried, gold coated, and mounted on stubs for SEM viewing. *n* = the number of seeds examined. Seed coat thickness was consistent in that the seed coat was thickest at the tapered end of the seed and thinner over the rest of the seed.

### Seed Morphology

Given our seed mass results, we expected to find morphological differences between seeds from taxa with contrasting mating systems. Contrary to these expectations, no differences in seed morphology were apparent between sister taxa. However, since we did not examine early seedlings and their cotyledons, we cannot eliminate the possibility of seedling morphological differences between the sister taxa. The considerable overlap in seed morphology and seed shape of members of these taxa indicates either no or weak selection on seed morphology or constraints limiting the traits' ability to diverge. We do not believe that lack of time is a factor. Given that the sister taxa have diverged in other features (e.g., seed mass, flower size, P : O ratio), sufficient time has obviously elapsed since the taxa split for seed morphological differences to evolve.

The seed morphology of *Clarkia unguiculata* and *Clarkia xantiana* supports their close taxonomic relationship, which was originally hypothesized by Lewis and Lewis (1955) on the basis of floral morphology. *Clarkia concinna* belongs to a different section of the genus and is thus more distantly related to the other species. In addition, its seeds differ in mass and morphology from the seeds of the other species and may be specialized for a different mode of dispersal (Snijman and Linder 1996; Garner and Witkowski 1997; Venable et al. 1998). All seeds of the *C. concinna* subspecies have elongated exotesta cells surrounding their truncated end, which might give these heavier seeds more lift for dispersal.

The classic *Clarkia* phylogeny by Lewis and Lewis (1955) has largely held true, but Systma and Smith (1988) have recently proposed several alterations based on DNA analysis. In their most parsimonious trees, *C. unguiculata* and *C. xantiana* do not form a monophyletic clade but are either (1) part of a larger *Clarkia* lineage in which all sections split at a polychotomous node or (2) aligned, respectively, with *Clarkia biloba* of section Sympherica and *Clarkia bottae* of section Fibula. The species' indistinguishable seeds support the first interpretation. In the Lewis and Lewis tree, *C. concinna* (section Eucharidium) is part of the same *Clarkia* lineage as *C. unguiculata* and *C. xantiana*, as opposed to the

Systma and Smith tree, in which *C. concinna* is removed from these sections and moved to a lineage within the section Godetia. Our seed data provided some support for the Systma and Smith tree.

The seeds of two other Onagraceae genera, *Oenothera* and *Ludwigia*, share several characteristics with the *Clarkia* taxa described here. They also lack an endosperm and have small seeds with a single-layered exotesta, a variably thick endotesta, and crystals in their seed coat (Tobe et al. 1987, 1988). In contrast to *Clarkia*, their crystals are in their endotesta and not the exotesta (Tobe et al. 1987, 1988). In *Phaseolus vulgaris* L., crystal growth is accompanied by a thickening of the cell walls, which provides the seed with a strong barrier against intrusive elements (e.g., bacteria) (Arnott and Webb 1983; Barnabas and Arnott 1990). The *Clarkia* crystals are in the thickest areas of the seed coat, and at these locations, the seed may have the most protection. The role of these crystals in seed protection or germination still needs to be elucidated.

### Conclusions

Apparently, the evolutionary forces that produced contrasting mating systems in *Clarkia* have resulted in changes in seed size and seed number but not seed coat morphology. The selfing taxa studied here produce many small seeds, possibly as an adaptive response to inhabiting more marginal, stressful environments than their outcrossing sister taxa. Evidence for a more extensive seed bank or greater seed dispersal for selfers would support this hypothesis.

Species identification cannot be made solely on the basis of seed coat morphology and sculpturing in this genus. The seed morphology of *Clarkia unguiculata* and *Clarkia xantiana* ssp. *xantiana*, along with their sister taxa, reflects their close evolutionary relationship, while that of the *Clarkia concinna* subspecies supports their current taxonomic position, which is more distant to the other species. Our studies of seed coat morphology support the current taxonomic sections in this genus. Additional studies of seed coat morphology in other *Clarkia* species may prove helpful in resolving the phylogeny of this genus.

### Acknowledgments

We thank Dr. S. Mazer for help in collecting seeds and for her knowledge of the genus. Comments by an anonymous re-

viewer greatly improved our presentation of this work and are gratefully acknowledged. This work was supported by the Senior Research Project Fund of Gettysburg College and National Science Foundation grant DEB 98-16256 to V. A. Delesalle.

### Literature Cited

- Allen GA, LD Gottlieb, VS Ford 1991 Electrophoretic evidence for the independent origins of two self-pollinating subspecies of *Clarkia concinna* (Onagraceae). *Can J Bot* 69:2299–2301.
- Andersson L, P Milberg, W Schutz, O Steinmetz 2002 Germination characteristics and emergence time of annual *Bromus* species of differing weediness in Sweden. *Weed Res* 42:135–147.
- Araki S, I Washitani 2000 Seed dormancy/germination traits of seven *Persicaria* species and their implication in soil seed-bank strategy. *Ecol Res* 15:33–46.
- Arnott HJ, MA Webb 1983 Twin crystals of calcium oxalate in the seed coat of the kidney bean. *Protoplasma* 114:23–34.
- Baker HG 1972 Seed weight in relation to environmental conditions in California. *Ecology* 53:997–1010.
- Barnabas AD, HJ Arnott 1990 Calcium oxalate crystal formation in the bean (*Phaseolus vulgaris* L.) seed coat. *Bot Gaz* 151:331–341.
- Bartholomew B, LC Eaton, PH Raven 1973 *Clarkia rubicunda*: a model of plant evolution in semiarid regions. *Evolution* 27:505–517.
- Bekker RM, JP Bakker, U Grandin, R Kalamees, P Milberg, P Poschlo, K Thompson, JH Willems 1998 Seed size, shape, and vertical distribution in the soil: indicators of seed longevity. *Funct Ecol* 12:834–842.
- Bobrov AVFCH, AP Melikian, EY Yembaturova 1999 Seed morphology, anatomy and ultrastructure of *Phyllocladus* L.C. & A. Rich. ex Mirb. (Phyllocladaceae (Pilg.) Bessey) in connection with the generic system and phylogeny. *Ann Bot* 83:601–618.
- Bowman RN 1987 *Clarkia concinna* subsp. *automixa* (Onagraceae): a new subspecies from the south bay region, central California. *Madroño* 34:41–47.
- Brown JS, DL Venable 1986 Evolutionary ecology of seed-bank annuals in temporally varying environment. *Am Nat* 127:31–47.
- Chance GD, JD Bacon 1984 Systematic implications of seed coat morphology in Nama (Hydrophyllaceae). *Am J Bot* 71:829–842.
- Chuang TI, R Ornduff 1992 Seed morphology and systematics of Menyanthaceae. *Am J Bot* 79:1396–1406.
- Eckhart VM, MA Geber 1999 Character variation and geographic range in *Clarkia xantiana* (Onagraceae): breeding system and phenology distinguish two common species. *Madroño* 46:117–125.
- Garner RD, ETF Witkowski 1997 Variations in seed size and shape in relation to depth of burial in the soil and pre-dispersal predation in *Acacia nilotica*, *A. tortilis* and *Dichrostachys cinerea*. *S Afr J Bot* 63:371–377.
- Gottlieb LD 1984 Electrophoretic analysis of the phylogeny of the self-pollinating populations of *Clarkia xantiana*. *Plant Syst Evol* 147:91–102.
- Grime JP 1977 Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111:1169–1194.
- Holtsford TP, NC Ellstrand 1992 Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* 46:216–225.
- Hufford L 1995 Seed morphology of Hydrangeaceae and its phylogenetic implications. *Int J Plant Sci* 156:555–580.
- Kamenetsky R, Y Gutterman 2000 Germination strategies of some *Allium* species of the subgenus *Melanocrommyum* from the arid zone of Central Asia. *J Arid Environ* 45:61–71.
- Kumar P, CD Rao, G Rajaseger, AN Rao 1999 Seed surface architecture and random amplified polymorphic DNA profiles of *Paulownia fortunei*, *P. tomentosa*, and their hybrid. *Ann Bot* 83:103–107.
- Leishman MR, M Westoby, E Jurado 1995 Correlates of seed size variation: a comparison among five temperate floras. *J Ecol* 83:517–529.
- Lewis H, ME Lewis 1955 The genus *Clarkia*. *Univ Calif Publ Bot* 20:241–392.
- Lewis H, PH Raven 1958 Rapid Evolution in *Clarkia*. *Evolution* 12:319–336.
- Matthews JF, PA Levine 1986 The systematic significance of seed morphology in *Portulaca* (Portulacaceae) under scanning electron microscopy. *Syst Bot* 11:302–308.
- Mazer S 1989 Ecological, taxonomic and life history correlates of seed mass among Indiana dune angiosperms. *Ecol Monogr* 59:153–175.
- McCue KA, TP Holtsford 1998 Seed bank influences on the genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *Am J Bot* 85:30–36.
- Murray DR 1984 The seed and survival. Pages 1–44 in DR Murray, ed. *Seed physiology*. Vol 2. Germination and reserve mobilization. Academic Press, Sydney.
- Raven PH 1988 Onagraceae as a model of plant evolution. Pages 85–107 in LD Gottlieb, SK Jain, eds. *Plant evolutionary biology*. Chapman & Hall, New York.
- Runions CJ, MA Geber 2000 Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. Size and development of floral organs. *Am J Bot* 87:1439–1451.
- Salisbury EJ 1942 The reproductive capacity of plants. Bell & Sons, London.
- Seavey SR, RE Magill, PH Raven 1977 Evolution of seed size, shape, and surface architecture in the tribe Epilobieae (Onagraceae). *Ann Mo Bot Gard* 64:18–47.
- Shetler SG, NR Morin 1986 Seed morphology in North American Campanulaceae. *Ann Mo Bot Gard* 73:653–688.
- Smith CC, SD Fretwell 1974 The optimal balance between the size and number of offspring. *Am Nat* 108:499–506.
- Snijman DA, HP Linder 1996 Phylogenetic relationships, seed characters, and dispersal system evolution in Amaryllideae (Amaryllidaceae). *Ann Mo Bot Gard* 83:362–386.
- Systma KJ, JF Smith 1988 DNA and morphology: comparisons in the Onagraceae. *Ann Mo Bot Gard* 75:1217–1237.
- Templeton AR, DA Levine 1979 Evolutionary consequences of seedbanks. *Am Nat* 114:232–249.
- Thompson K, SR Band, JG Hodgson 1993 Seed size and shape predict persistence in soil. *Funct Ecol* 7:236–241.
- Thompson K, DJ Hodgson 1988 Seed mass, habit, and life history: a re-analysis of Salisbury (1942, 1974). *New Phytol* 138:163–166.
- Tobe H, PH Raven 1986 A comparative study of the embryology of *Ludwigia* (Onagraceae): characteristics, variation, and relationships. *Ann Mo Bot Gard* 73:768–787.
- Tobe H, PH Raven, C Peng 1988 Seed coat anatomy and relationships of *Ludwigia* sects. *Microparium*, *Dantia*, and *Miquelia* (Onagraceae), and notes on fossil seeds of *Ludwigia* from Europe. *Bot Gaz* 149:450–457.
- Tobe H, WL Wagner, H Chin 1987 A systematic and evolutionary study of *Oenothera* (Onagraceae): seed coat anatomy. *Bot Gaz* 148:235–257.

- Tonsor SJ, S Kalisz, J Fisher, TP Holtsford 1993 A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. *Evolution* 47:833–843.
- Vasek FC, J Harding 1976 Outcrossing in natural populations. V. Analysis of outcrossing, inbreeding, and selection in *Clarkia exilis* and *Clarkia tembloriensis*. *Evolution* 30:403–411.
- Vasek FC, V Weng 1988 Breeding systems of *Clarkia* sect. *Phaeostoma* (Onagraceae). I. Pollen-ovule ratios. *Syst Bot* 13: 336–350.
- Venable DL, JS Brown 1988 The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in a variable environment. *Am Nat* 131:360–384.
- Venable DL, E Dyreson, D Pinero, JX Becerra 1998 Seed morphometrics and adaptive geographic differentiation. *Evolution* 52: 344–354.
- Wulff RD 1986 Seed size variation in *Desmodium paniculatum*. II. Effects on seedling growth and physiological performance. *J Ecol* 74:99–114.