PHYSIOLOGICAL PERFORMANCE IN *CLARKIA* SISTER TAXA WITH CONTRASTING MATING SYSTEMS: DO EARLY-FLOWERING AUTOGAMOUS TAXA AVOID WATER STRESS RELATIVE TO THEIR POLLINATOR-DEPENDENT COUNTERPARTS?

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Consistent differences in the physiological performance of wild populations of closely related plant taxa may be the result of environmentally induced phenotypic plasticity or adaptive evolution (or a combination of the two). Here we report the results of a field study of physiological and fitness-related traits in geographically proximate sister taxa in the annual wildflower genus *Clarkia* (Onagraceae) and interpret the differences between them in light of their ecological and reproductive differences. Within two pairs of taxa, the predominantly autogamous (self-fertilizing) taxon flowers and completes its life cycle before its pollinator-dependent (predominantly outcrossing) counterpart growing in sympatry or at similar elevations in the southern Sierra Nevada. Selfers generally exhibited higher rates of photosynthesis and transpiration than their outcrossing sister taxa, and, except for the earliest-flowering (autogamous) taxon, both photosynthetic and transpiration rates tended to decline as the season progressed. Within taxa, high photosynthetic rates were positively correlated with lifetime fruit production, and selfers had lifetime fruit production equivalent to or higher than that of outcrossers, despite the fact that the latter had higher aboveground stem biomass. These patterns are consistent with the hypothesis that natural selection has favored higher gas exchange rates in selfers to allow them to achieve their faster life cycles and so escape seasonal late-spring drought. An alternative explanation is that the differences in gas exchange rates represent environmentally induced plastic responses to the cooler temperatures and higher soil moisture content in early spring. Further experimental work is necessary to distinguish between these hypotheses.

Keywords: *Clarkia*, comparative physiology, life-history evolution, mating system, photosynthesis, transpiration, water use efficiency.

Online enhancement: appendix figures.

Introduction

Evolutionary transitions between pollinator-independent self-fertilization and vector-mediated outcrossing are common among angiosperms, with more than 20% of flowering-plant taxa regularly self-fertilizing (Stebbins 1974). Most research has attempted to explain the evolution of selfing as an adaptive response to selection, that is, as a strategy that in certain environments provides fitness advantages that outweigh the disadvantages of inbreeding depression and reduced genetic diversity in the offspring generation. Advantages of autogamous (within-flower) self-fertilization include guaranteed pollination ("reproductive assurance") where pollinators are scarce or competition among individual plants for pollinator visits is intense, the transmission of alleles through both selfing and outcrossing (for mixed mating systems), the opportunity to eliminate resource allocation to secondary sexual traits, and the opportunity to complete the life cycle rapidly

in order to avoid floral predators, herbivores, or water stress (Fisher 1941; Stebbins 1957; Antonovics 1968; Jain 1976; Lloyd 1980, 1992; Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Hamilton et al. 1990; Schoen and Brown 1991; Yahara 1992; Jarne and Charlesworth 1993; Holsinger 1996; Schoen et al. 1996; Byers and Waller 1999; Fishman and Wyatt 1999; Aarssen 2000; Kalisz and Vogler 2003; Kalisz et al. 2004; Moeller and Geber 2005; Snell and Aarssen 2005; Goodwillie et al. 2010; Wu et al. 2010; but see Herlihy and Eckert 2002).

Support for the reproductive-assurance hypothesis comes from studies showing that selfers often occupy habitats where pollinators may be absent or unreliable, such as the margins of species' geographic ranges (Baker 1955; Jain 1976; Eckhart and Geber 1999; Busch 2005; Geber and Eckhart 2005; Brunet and Sweet 2006*a*; Geber and Moeller 2007; but see Herlihy and Eckert 2005; Brunet and Sweet 2006*b*). There is still uncertainty, however, about whether the other selective factors described above are also important or context specific (e.g., Barrett 1995; Barrett and Harder 1996; Cheptou and Dieckmann 2002; Herlihy and Eckert

2004; Ivey and Carr 2005; Steets et al. 2007; see Snell and Aarssen 2005 for corroborating evidence). These alternative explanations for the evolution of selfing are not mutually exclusive.

Autogamously selfing taxa often differ from their pollinator-dependent (and typically outcrossing) relatives in a number of traits in addition to their mating system: the former often flower and senesce earlier, produce sequential flowers faster, produce smaller flower displays, or display smaller and shorter-lived flowers (see table 1 for examples in *Clarkia*; for evaluations of other taxa, see Armbruster et al. 2002; Goodwillie et al. 2010; Theiss et al. 2010; Wu et al. 2010). These observations suggest that the evolution of selfing may in some cases be affected by the same ecological factors that influence the evolution of these life-history and morphological traits.

One of the most important such factors is water availability, which has a major influence on the distribution and abundance of plant species (Cornwell and Grubb 2003), on plant productivity (Leith and Whittaker 1975), and on a variety of plant morphological, physiological, and life-history traits (Mooney and Dunn 1970; Ehleringer 1975; Givnish 1979, 1986; Ludlow 1989; Geber and Dawson 1990, 1997; Chapin et al. 1993; Dudley 1996a, 1996b; Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffin 2003). Comparative studies have led to the proposition that plants experiencing seasonal drought have evolved one of two alternative functional strategies for dealing with annually predictable reductions in water availability: drought escape or dehydration avoidance (Ludlow 1989). Drought escape is characterized by rapid growth and completion of the life cycle before the onset of severe drought, through the maintenance of high metabolic rates when water is relatively abundant (Mooney et al. 1976; Geber and Dawson 1990, 1997; Aarssen 2000, 2008; Arntz and Delph 2001; McKay et al. 2003). Dehydration avoidance is characterized by slower growth, longer life cycles, and the maintenance of lower metabolic rates in order to reduce transpirational water loss (Geber and Dawson 1990, 1997; Chapin et al. 1993; Stanton et al. 2000; Arntz and Delph 2001; McKay et al. 2003). In theory, a drought-escape strategy should favor plants with high stomatal conductance, because this would allow them to maintain a high photosynthetic rate, albeit at the potential cost of a high transpiration rate and low water use efficiency (WUE). Conversely, a dehydration-avoidance strategy should favor plants with low stomatal conductance in order to reduce transpirational water losses, with a consequent increase in WUE but at the cost of a reduction in photosynthetic rate (Cohen 1970; Cowan 1986; Givnish 1986; Schultze et al. 1987). Recent detailed studies of phenotypic selection within a variety of plant species have provided partial support for these adaptive scenarios, although their predictions have not always been fully supported (Dudley 1996a, 1996b; Heschel et al. 2002, 2004; Ludwig et al. 2004; Casper et al. 2005; Heschel and Riginos 2005; Sherrard and Maherali 2006; Donovan et al. 2007, 2009; Wu et al. 2010).

For drought-escaping annuals, a short life cycle would be facilitated by the production of smaller, more rapidly developing flowers (Moore and Lewis 1965; Small 1972; Guerrant 1982, 1989; Diggle 1992; Fenster et al. 1995; Andersson

1997; Geber and Dawson 1997; Aarssen 2000; Ackerly et al. 2000; Armbruster et al. 2002; Heschel and Riginos 2005), which in turn may have elevated selfing rates if they exhibit sufficiently less dichogamy or herkogamy than relatively slow-developing flowers. Under this scenario, selfing might evolve as an indirect effect of selection favoring accelerated floral development or a compressed life history if traits influencing mating system are genetically correlated with those influencing development rates, even if selfing itself is not advantageous. Autogamous selfing might also be under direct selection for reasons of reproductive assurance and might co-evolve with early flowering if competition for pollinator visitation is particularly intense relatively early in the season. This could be the case if the species diversity of flowering plants peaks during this time or if pollinator abundance is low.

Seasonal drought is characteristic of the habitats occupied by many Californian populations of the genus *Clarkia*, which in the Sierra Nevada flowers relatively late in the spring as soils are rapidly drying (hence the genus's common name, Farewell to Spring). In addition, *Clarkia* taxa differ in their flowering phenology and habitat distribution in ways that are likely to have a major effect on their ability to access soil moisture, with the result that selection may favor drought escape in some taxa and dehydration avoidance in others. Moreover, selection on ecophysiological traits may be correlated with selection on mating system and life history, for the reasons outlined above and described in more detail below.

In this study, we investigated whether early- and late-flowering *Clarkia* taxa have adopted different physiological strategies for dealing with seasonal drought. In two pairs of sister taxa, each consisting of one pollinator-dependent and one highly autogamous (pollinator-independent) taxon, the latter flowers earlier and produces smaller and more rapidly developing flowers than its predominantly outcrossing counterpart. If rapid development and early flowering enable pollinator-independent populations to avoid water stress by completing their life cycle while soil moisture is relatively high, then these populations may be able to tolerate higher transpiration rates and achieve faster photosynthetic rates than their outcrossing counterparts. Moreover, if higher instantaneous photosynthetic rates confer higher individual fitness, then the relationship between photosynthetic rate and lifetime fruit production should be positive within populations of both pollinator-independent and pollinator-dependent taxa. Annual plants can allocate between 15% and 30% of lifetime carbon gain to reproduction (Mooney 1972), so higher photosynthetic rates may well contribute significantly to individual fitness. In the work reported here, we address the following questions and predictions.

1. How do environmental conditions differ among sites occupied by autogamous versus pollinator-dependent taxa throughout the growing season? We predict that the relatively early-flowering and highly autogamous (pollinator-independent) taxa will flower under conditions of greater water availability than their later-flowering, pollinator-dependent counterparts.

2. Do autogamous and pollinator-dependent taxa differ physiologically? We predict that autogamous taxa will exhibit higher gas exchange rates or lower instantaneous WUE (WUE_i), either as plastic responses to cooler conditions and

Table 1

Differences in Life History and Mating System–Related Traits between Predominantly Outcrossing and Autogamously Selfing Sister Taxa: *unguiculata* (Outcrosser) versus *exilis* (Selfer) and *xantiana* (Outcrosser) versus *parviflora* (Selfer)

	exilis	unguiculata	P, reference	parviflora	xantiana	P, reference
Life-history and developmental traits:						
Days from germination to first flower in greenhouse	72.09 (.36)	73.14 (.49)	ns; Dudley et al. 2007	51.90 (.53)	62.48 (.47)	.031; Dudley et al. 2007
				27.02 (1.3),	33.77 (.54),	ns; Mazer et al. 2004 (two replicates)
Days to first flower in field				81.68 (2.3),	90.77 (2.4)	<.001; Eckhart et al. 2004
				150–190	175–210	
Days from bud break to anther dehiscence	2.34 (.04)	4.32 (.08)	.009; Dudley et al. 2007	.96 (.02)	2.80 (.04)	.0008; Dudley et al. 2007
				1.48 (.13),	4.72 (.17),	.0001; Mazer et al. 2004 (two replicates)
				1.83 (.07)	3.14 (.15)	
Days from bud break to stigma receptivity	1.59 (.05)	7.33 (.12)	.0007; Dudley et al. 2007	.48 (.04)	4.19 (.06)	.004; Dudley et al. 2007
				2.77 (.16),	5.71 (.15),	.0001; Mazer et al. 2004 (two replicates)
				2.99 (.24)	7.86 (.39)	
Rate of flower production (days from first to sixth flower)	5.3 (.1)	6.4 (.1)	.059; Dudley et al. 2007			
Flower plastochron (days between successive flower production)				.76 (.03)	1.05 (.03)	<.05; Runions and Geber 2000
Pollen : ovule ratio:						
In field populations	42.07–47.01	96.30–118.42	<.0001; Delesalle et al. 2008	56.66–76.23	118.67–127.75	<.0001; Delesalle et al. 2008
In greenhouse[b]	E: 31.2 (.45)	E: 85.2(1.34)	.0004; Mazer et al. 2009	E: 31.2 (1.39)	E: 48.6 (1.57)	.013; Mazer et al. 2009
	L: 23.7 (.42)	L: 59.4 (1.18)	.0006; Mazer et al. 2009	L: 31.3 (1.76)	L: 55.6 (2.21)	.010; Mazer et al. 2009
Mating system:						
λ (outcrossing rate)	.440 ± .036,	.96	Vasek and Harding 1976[a] (three populations)			
	.378 ± .054,					
	.894 ± .041		Vasek 1967[a]			
	.43		Vasek 1965[a]			
	.03–.70		Vasek 1964b[a]			
Seeds/capsule in the absence of pollinators	60.9 (range: 19–89)	20.6 (range: 2–57)	Vasek 1958[a]	93.1 (range: 58–100)	9.7 (range: 0–40.0)	<.0001; Moeller 2006
Autogamous fruit set (%)				.32 (.41)	3.29 (1.61)	<.0001; Moeller 2006
Herkogamy (mm)	−.76 (.05)	3.01 (.09)	.0004; Dudley et al. 2007	−.48 (.03)	1.39 (.05)	.012; Dudley et al. 2007
Duration of protandry (days)				1.33 (.18)	3.15 (.32)	.0001; Mazer et al. 2004
				1.16 (.25)	2.55 (.17)	
				1.97 (.07)	6.89 (.12)	<.05; Runions and Geber 2000
				.19 (.68)	3.93 (1.16)	<.0001; Moeller 2006

Note. Values reported are either (1) trait means with standard errors indicated in parentheses (1 SE) or standard deviations indicated as ± 1 SD or (2) ranges of means observed in each taxon. Underlining indicates P values < 0.05, i.e., that an experiment found that sister taxa differ with respect to trait means. NS = no significant difference detected between sister taxa.

[a] Study reported phenotypic ranges or did not directly compare sister taxa (no P values available).

[b] E = early flowers; L = late flowers.

greater soil moisture or as an adaptive strategy to permit drought escape by developing and maturing rapidly.

3. Do physiological rates change as the season progresses, and if so, do such changes appear to be adaptive? We predict that plants will express more drought-avoidance strategies as they mature than at earlier stages of development (Clarke and Durley 1981). We predict, in addition, that among populations of our focal pollinator-dependent *Clarkia* taxa (which flower relatively late and are longer-lived than their autogamous sister taxa), WUE_i should increase between preflowering and flowering life stages as water availability declines and evaporative water loss through petals becomes a significant factor (see Galen 2006). An alternative prediction is that if *Clarkia* experiences strong belowground interspecific competition, then low WUE might be advantageous as soil moisture declines if high rates of evapotranspiration and soil moisture uptake reduce the fitness of competitors more than they place a focal plant at risk.

4. Do autogamous and pollinator-dependent taxa differ in the direction or magnitude of temporal change in their physiological rates across their life cycles? We predict that if pollinator-dependent taxa experience more acute seasonal increases in water stress than their early-flowering autogamous counterparts, they might also show greater seasonal changes in gas exchange rates and increases in WUE_i.

5. Are rapid rates of photosynthesis associated with higher reproductive output among individuals? If so, natural selection may favor more rapid photosynthesis in both autogamous and pollinator-dependent populations, but the cost of high transpiration (excessive water loss) may not permit its expression in the relatively late-flowering populations of the latter.

Methods

Study Species

Clarkia (Onagraceae) is a genus of ~41 self-compatible, winter annual, herbaceous taxa. Phylogenetic relationships inferred from molecular variation (Lewis and Lewis 1955; Gottlieb 1984, 1988; Sytsma and Gottlieb 1986; Sytsma 1990; Sytsma et al. 1990; Gottlieb and Ford 1996) indicate that autogamous self-fertilization has evolved independently in the genus multiple times. Accordingly, *Clarkia* has been the subject of many studies evaluating the causes and consequences of the evolution of contrasting mating systems (e.g., Moore and Lewis 1965; Vasek and Harding 1976; Holtsford and Ellstrand 1992; Runions and Geber 2000; Fausto et al. 2001; Eckhart et al. 2004; Mazer et al. 2004, 2007; Geber and Eckhart 2005; Moeller and Geber 2005; Moeller 2006). As in many taxa, the degree of autogamy is determined by the degree of herkogamy and dichogamy exhibited by individual flowers (Lewis 1953; Vasek 1964a, 1965, 1967; Vasek and Harding 1976; Holtsford and Ellstrand 1992).

Here we focus on two pairs of diploid taxa, where each pair has diverged in life history and the degree to which they depend on pollinators to set seed. The first pair includes the pollinator-dependent *C. unguiculata* Lindley and the facultatively autogamously selfing *C. exilis* Lewis and Vasek; the second includes two subspecies of *C. xantiana* Gray: the pollinator-dependent ssp. *xantiana* and the autogamous ssp. *parviflora* (Lewis and Raven 1992). From this point on, we refer to these taxa as *unguiculata*, *exilis*, *xantiana*, and *parviflora*, respectively.

In both sister pairs, selfing is proposed to be the derived state (Vasek 1964b, 1977; Gottlieb 1984) and is associated with evolutionary shifts in phenology, flower size and longevity, floral development rates, and habitat preferences (Vasek 1958, 1965, 1967, 1971, 1977; Vasek and Sauer 1971; Vasek and Harding 1976; Eckhart and Geber 1999; Dudley et al. 2007). Under greenhouse and field conditions, the flowers of *xantiana* and *unguiculata* develop more slowly, remain fresh and unwilted longer, and are more herkogamous and dichogamous than those of their selfing sister taxa (Vasek 1958; Dudley et al. 2007). In addition, under pollinator-free conditions, *exilis* and *parviflora* achieve nearly 100% autogamous fruit set; fruit set is typically less than 10% in greenhouse-raised outcrossing *unguiculata* and *xantiana* (S. J. Mazer, V. A. Delesalle, L. S. Dudley, and H. Paz, personal observations). In general, the selfing taxa consist of individuals that flower earlier than and complete their life cycles before their pollinator-dependent counterparts. These differences are observed both under greenhouse conditions and in wild populations growing sympatrically or at similar elevations (Vasek 1958, 1964a, 1967, 1977; Eckhart and Geber 1999; Runions and Geber 2000; Geber and Eckhart 2005; Dudley et al. 2007).

Estimated outcrossing rates are generally consistent with these morphological and developmental differences, although populations of *exilis* exhibit highly variable outcrossing rates (population means range from 0.03 to 0.89; table 1; Vasek 1964b, 1965, 1967; Vasek and Harding 1976; Vasek and Weng 1988). In addition, a greenhouse experiment conducted across five watering treatments found that outcrossing *unguiculata* (but not *exilis*) exhibited significant inbreeding depression in fitness-related traits under most conditions (Lowry 2007). This difference suggests that *exilis* has had a history of recurrent selfing that has purged populations of deleterious recessives. Published estimates of outcrossing rates in field populations of *xantiana* are unavailable, but ecological studies and allozyme surveys have shown that *xantiana* populations require insect pollination to achieve high levels of fruit set and are genetically highly polymorphic, while populations of *parviflora* are autogamous and monomorphic (Gottlieb 1984; Moeller and Geber 2005; Moeller 2006).

Unguiculata occupies a broad geographic range on woodland slopes, grasslands, and road cuts in the Coast Ranges; in the southern and western foothills of the Sierra Nevada; and in the Tehachapi, Western Transverse, Peninsular, and South Coast ranges. *Exilis* is much less common and is restricted to vegetation types similar to those of *unguiculata* in and near the Kern River Valley (Kern and Tulare counties, California; Hickman 1993). Where both species coexist, *exilis* tends to occupy the edges of boulders and streambeds, where soil may retain more moisture than the exposed slopes often occupied by *unguiculata*. The outcrossing and relatively widespread *xantiana* occupies rocky hillsides in the southern Sierra Nevada, the Tehachapi Mountains, and the Western Transverse Ranges; *parviflora* is typically restricted to rocky slopes in the eastern portion of the species' range (Hickman 1993).

Dudley et al. (2007) and Delesalle et al. (2008) provide additional information about these four taxa.

Field Survey: Environmental Attributes

This study was conducted in the region of Lake Isabella in the southern Sierra Nevada of California across an elevation gradient ranging from 430 to 1607 m. At each of 12 sites (table 2) where one or more of the focal taxa occurs, an environmental sensor was placed in February 2008 to record air temperature, relative humidity, precipitation, and soil temperature (we used Em50 data loggers with ECH20 rain gauges, 5TM soil temperature sensors, and air temperature sensors; Decagon Devices, Pullman, WA; table 2 indicates which of our focal taxa occur regularly at each site). For each site, a drought index (DI) was calculated each month of the growing season as mean daily ambient temperature per month (°C) minus one-third of the total monthly precipitation (mm), with temperatures of <0°C changed to 0°C before calculation (Walter 1973). For each taxon, the mean DI was calculated across the sites in which it occurred (for example, the *exilis* DI was averaged across two sites, while that for *unguiculata* was averaged across five sites; table 2).

Gas Exchange Measurements

A portable infrared gas exchange analyzer (IRGA; LiCor 6400, Lincoln, NE) was used to measure photosynthetic rate (A), transpiration rate (E), and stomatal conductance (g_s) on individual leaves under field conditions between 0900 and 1200 hours; leaf and air temperature were also recorded at the time of measurement. WUE_i was calculated as A/E and therefore represents the leaf's instantaneous WUE. Given that A and E can change within minutes in response to water stress (Larcher 1995), WUE_i is a good measure of a plant's performance under the conditions at the time of measurement. LiCor settings used were as follows: light source, 6400-40 fluorometer; stability, measurements of CO_2 and H_2O in the sample chamber and flow had to be stable for 15 s with a change in slope of <1; stomatal ratio = 0.5; flow = 500 μmol s^{-1}; PAR_i (PAR in the IRGA) = 600. Sampled leaves were always taken from the main stem, and their node position (relative to the first true leaf) on the stem was recorded.

In cases where leaves were too narrow to fill the area of the LiCor 6400's 2-cm^2 leaf chamber, the leaf area exposed in the chamber was calculated manually. This was accomplished by tracing the edges of the portion of the leaf inside the chamber with a permanent ink marker, taping the leaf to a piece of paper with transparent tape, and then scanning it with a digital scanner. We then used the image analysis program ImageJ (NIH, available at http://rsb.info.nih.gov/ij/index.html) to determine the measured area of each leaf and applied the LiCor's recomputation utility to adjust each gas exchange rate to reflect the actual leaf area contained within the chamber.

No Effects of Leaf Removal on Physiological Rates

In the field, plants are distributed on steep and often unstable slopes, often making it unfeasible to carry the IRGA from plant to plant. Thus, physiological parameters were measured on single excised healthy leaves taken from the primary stem of each plant. Each leaf was clamped into the chamber of the IRGA within 30 s of excision, and physiological parameters were recorded within 1–2 min, at which point the measurements were stable.

In December 2008, we conducted a greenhouse experiment to determine whether leaf excision significantly altered leaf physiological performance. Ten individuals of *xantiana* were raised under greenhouse conditions. After flowering had started, one leaf per plant was selected, and its physiological performance was recorded for 5 min before excision; then the leaf was excised, and recordings were continued for an additional 5 min. A repeated-measures analysis was con-

Table 2

Location and Elevations of Sites Where Environmental Sensors Were Placed, with the Taxa That Regularly Occur at Each Site

Locality name	Elevation (m)	Latitude (N)	Longitude (W)	*exilis*	*unguiculata*	*xantiana*	*parviflora*
Stark Creek	457	35°28.44′	118°43.53′	X	X		
Live Oak	475	35°28.75′	118°44.91′	X	X	X	
Kern Canyon Road	717	35°33.48′	118°34.76′		X	X	
Mill Creek	746	35°32.24′	118°36.84′		X	X	
Greenhorn Mountain Road	1205	35°43.29′	118°30.04′		X	X	
Borel Road	707	35°35.04′	118°31.30′			X	
Camp 3	896	35°48.69′	118°27.20′			X	X
Sawmill Road	932	35°40.90′	118°28.52′			X	X
Sherman Pass	1335	35°58.73′	118°28.05′			X	
Johnsondale Bridge	1157	35°58.22′	118°29.10′				X
Chimney Peak	1451	35°46.35′	118°05.19′				X
Long Valley[a]	1628	35°48.91′	118°05.45′				X

Note. Mean drought indices experienced by each taxon were based on their occurrences at sites with environmental sensors. An X indicates that the taxon occurs at the site. Not all taxa at each site were surveyed for their physiological performance (see table 4 for surveyed sites and taxa).

[a] The Long Valley population has attributes of both *xantiana* and *parviflora*. Its elevation, flowering time, and flower color polymorphism are more similar to *parviflora*, but its petals are intermediate in size between those of typical *xantiana* and *parviflora* populations, and its flowers display high herkogamy and dichogamy.

ducted to detect significant differences among plants, the effect of leaf status (attached vs. excised), and plant × leaf status interaction effects on photosynthesis, conductance, transpiration, and WUE$_i$. There was no significant effect of leaf excision on any physiological trait measured during the 5 min after excision (table 3); averaged across the 10 study plants, excision resulted in changes in mean physiological rates of less than 2.5%. Significant leaf status × time interaction was detected for conductance and transpiration rates, indicating that excision may have influenced their stability.

Visual inspection of the data showed that the absolute effect was very small and limited to the rate measured immediately after excision. One minute after excision, conductance and transpiration rates equaled those measured before excision.

Field Survey: Physiological Performance Before and During Flowering

Gas exchange measurements were conducted on plants selected in each field population on two sampling dates: be-

Table 3

Results of Repeated-Measures Analysis to Detect Effects of Plant, Leaf Status (Attached vs. Excised), and Plant × Leaf Status Interactions on Physiological Performance under Greenhouse Conditions

	F or Pillai's trace value	df in numerator	df in denominator	Exact or approximate F	P
Photosynthesis (A; μmol CO_2 m^{-2} s^{-1}):					
Between subjects:					
All	43.33	10	9	38.99	<.0001
Intercept	375.61	1	9	3380.45	<.0001
Plant ID	43.28	9	9	43.28	<.0001
Leaf status	.05	1	9	.41	.5374
Within subjects:					
All	1.60	40	36	.60	.9399
Time	.41	4	6	.61	.6698
Time × plant ID	1.32	36	36	.49	.9821
Time × leaf status	.82	4	6	1.23	.3881
Conductance (g_s; mol m^{-2} s^{-1}):					
Between subjects:					
All	134.84	10	9	121.36	<.0001
Intercept	1685.92	1	9	15173	<.0001
Plant ID	134.84	9	9	134.84	<.0001
Leaf status	.0014	1	9	.01	.9127
Within subjects:					
All	2.26	40	36	1.17	.3214
Time	.77	4	6	1.16	.4139
Time × plant ID	2.07	36	36	1.07	.4231
Time × leaf status	3.34	4	6	5.01	.0405
Transpiration (E; mol H_2O m^{-2} s^{-1}):					
Between subjects:					
All	114.48	10	9	103.03	<.0001
Intercept	2657.87	1	9	23921	<.0001
Plant ID	114.46	9	9	114.46	<.0001
Leaf status	.02	1	9	.18	.6803
Within subjects:					
All	2.24	40	36	1.14	.3424
Time	.62	4	6	.93	.5055
Time × plant ID	2.00	36	36	1.00	.4959
Time × leaf status	3.47	4	6	5.20	.0373
WUE$_i$ (A/E; μmol mmol^{-1}):					
Between subjects:					
All	25.93	10	9	23.34	<.0001
Intercept	258.92	1	9	2330.31	<.0001
Plant ID	25.90	9	9	25.90	<.0001
Leaf status	.03	1	9	.27	.6189
Within subjects:					
All	1.77	40	36	.71	.8502
Time	.46	4	6	.69	.6257
Time × plant ID	1.40	36	36	.54	.9662
Time × leaf status	1.07	4	6	1.61	.2871

Note. The time effect detects variation across the five sequential measurements recorded on each attached and excised leaf. Pillai's trace test was used to detect all within-subject effects because it is considered to have the highest power (JMP 7.0 user's manual); this test provides approximate F values. WUE$_i$ = instantaneous water-use efficiency. Underlining indicates P values < 0.05.

fore and during flowering. Each sampling date was classified as either preflowering (sampled plants were not in flower) or flowering (populations were in flower, with all sampled plants producing buds and >60% of sampled plants bearing flowers and fruits). At each sampling period, one to four 20–50-m transects were established, and at randomly determined points along each transect one to three individuals were selected and labeled (*Clarkia* individuals are patchily distributed within populations, so sampling could not be spatially homogeneous). We aimed to measure every plant during both time periods, but many individuals died before flowering. Thus, to maintain similar sample sizes at each life stage, plants that died were replaced with others when the population was surveyed during flowering. Consequently, some flowering individuals were measured both before and during flowering. Sampling intensity and flowering status of the surveyed populations are shown in table 4.

Determining Final Plant Size and Fitness

Sampled plants were collected from the field after senescence in order to estimate final plant size and individual maternal fitness. Plants were gently pulled from the soil, placed in paper bags, and fully air-dried at room temperature. Leaves, roots, and fruits were removed, and the aboveground stem biomass was weighed to the nearest 0.1 mg. Attached filled fruits and peduncles (included to account for fruits missing because of herbivory) were counted to estimate lifetime fruit production. Fruits dehisce while and after plants naturally senesce, so collected plants included

different proportions of fruits that were filled with seeds. Consequently, it was not appropriate to use total aboveground biomass (including fruits and seeds) or fruit mass as an estimate of lifetime fitness. Aboveground stem biomass is strongly positively correlated with lifetime flower production (Mazer and Dawson 2001), so the combination of stem biomass and lifetime fruit production provides a reasonable estimate of reproductive output through both pollen and seeds.

Data Sets and Statistical Analyses

The Effects of Leaf Node Position and Leaf Temperature

Leaf temperature (T_{leaf}) and leaf node position on the primary stem influenced all physiological traits (see the appendix in the online edition of the *International Journal of Plant Sciences*). Thus, for all analyses, we controlled statistically for the effects of these factors by analyzing the residuals of each gas exchange parameter regressed on both T_{leaf} and leaf node position.

Detecting Physiological Differences between Sister Taxa

Because some individuals were sampled during both time periods, preflowering and flowering samples were analyzed separately to ensure that data points were independent. Residuals were calculated for each taxon pair (using pooled data from both sampling dates). All variables were checked for normality and, where warranted, $\log_{10}$ transformed to meet model assumptions of normality and/or homoscedas-

Table 4

Elevation, Location, and Sampling Information for the Four *Clarkia* Taxa Surveyed in 2008

Population	Preflowering		Flowering		Elevation (m)	GPS coordinates	
	Sampling date (2008)	n	Sampling date (2008)	n		Latitude (N)	Longitude (W)
C. unguiculata (pollinator-dependent):							
Cow Flat Road	March 26	39			660	35°31.79′	118°39.19′
Granite Road	April 10	44			869	35°41.45′	118°43.91′
Jack and Stage	March 11	44	May 19	56	1006	35°47.74′	118°42.15′
Live Oak	March 23	40	May 6	45	430	35°28.81′	118°44.89′
Stark Creek	March 24	43	May 7	24	443	35°28.26′	118°43.54′
C. exilis (autogamous, selfing):							
Granite Station	March 28	31	April 20	22	543	35°37.02′	118°51.53′
Stark Creek	March 24	41	April 23	41	443	35°28.26′	118°43.54′
Willow Spring	March 31	43	April 22	43	365	35°40.22′	118°54.12′
Woody Road	April 19	43	April 7	42	493	35°41.58′	118°51.95′
C. xantiana ssp. *xantiana* (pollinator-dependent):							
Borel Road	April 7	42	May 16, 17, 30	97	707	35°35.04′	118°32.30′
Camp 3	March 27	43	May 18	41	863	35°48.65′	118°27.24′
Greenhorn Mountain Road	April 8	43	June 9	37	1139	35°43.26′	118°30.04′
Live Oak	March 25	40			430	35°28.81′	118°44.89′
Sawmill Road	March 29	40			902	35°40.75′	118°28.45′
C. xantiana ssp. *parviflora* (autogamous, selfing):							
Chimney Peak	April 6	44	June 1	30	1334	35°46.33′	118°05.17′
Long Valley	April 24	45	June 7, 8	80	1607	35°48.53′	118°05.33′
Wofford Heights	April 21	50	May 8	61	859	35°41.35′	118°27.99′

Note. Underlining indicates sites where environmental sensors were located. GPS coordinates for sites appearing in table 2 differ slightly from those in this table when the sensor was placed a short distance from the sampled portion of the population.

ticity. Mixed-model ANOVAs (JMP, ver. 7.0) were conducted to detect significant differences between taxa (treated as fixed effects) and among populations (nested within species and treated as a random effect) with respect to all physiological traits. These ANOVAs detected no significant differences among population means, so population was excluded from subsequent models. Thus, we used a fixed-effect model to detect significant differences between sister taxa in each sampling period for each of the physiological traits recorded.

Detecting Temporal Changes in Physiological Performance within Each Taxon

Four data sets (one per taxon) were constructed to evaluate temporal change between preflowering and flowering sample dates within each taxon. Within each of the four taxa, data were pooled from all populations and both sampling dates, but to ensure independence of the data, plants that had been measured twice were excluded from the flowering-population samples. Residuals were calculated within each taxon before analysis. In *exilis*, three preflowering populations ($n = 31$–43 plants per population) and three flowering populations ($n = 13$–44) were included. In *unguiculata*, five preflowering populations ($n = 40$–44) and two flowering populations ($n = 13$–44) were included. In *parviflora*, three preflowering populations ($n = 44$–50) and three flowering populations ($n = 15$–40) were included. In *xantiana*, five preflowering populations ($n = 40$–43) and three flowering populations ($n = 37$–66) were included.

Mixed-model ANOVAs were conducted to detect significant effects of life stage (preflowering vs. flowering) and population (nested within life stage and treated as a random effect) with respect to each physiological trait. For these analyses, raw values (transpiration and WUE_i were log_{10} transformed) were analyzed in addition to the residuals of each trait on T_{leaf} and node position. Temporal changes in raw values were of interest because of their association with seasonal changes in air temperature and developmental stage (discussed below). These ANOVAs detected no significant differences among population means, and so population was excluded from the model. Fixed-effect ANOVAs were then conducted to determine whether life stages differed with respect to each of the gas exchange traits recorded.

Relationship between photosynthetic rate and lifetime fruit production. Within each taxon, the effect of photosynthetic rate on individual fitness was estimated by conducting a linear regression of lifetime fruit production on photosynthetic rate (controlling for T_{leaf} and node number by using residuals, as described above).

Differences between sister taxa in stem biomass, the total number of reproductive units, and lifetime fruit production. Within each sister pair, we compared aboveground stem biomass and lifetime fruit production, using all plants sampled during flowering. We conducted a mixed-model ANOVA to detect the effect of taxon and population (nested within taxon, as a random effect) on these fitness-related traits (both traits were log_{10} transformed). The population effect was not significant in any of the analyses and so was excluded from the final models.

Results

Field Survey: Environmental Attributes

Within the *exilis/unguiculata* sister pair, the sampled *exilis* populations flowered in April 2008, 2–4 wk earlier than *unguiculata* populations at similar elevations (table 4). Accordingly, *exilis* was found flowering at sites when the mean monthly DI indicated cooler temperatures and/or higher rainfall than was observed in May at the sites where *unguiculata* was flowering (fig. 1). The *parviflora/xantiana* pair exhibited a similar phenological difference when elevation was taken into account. For example, the Wofford Heights population of *parviflora* (at 859 m) flowered 8–10 d earlier than the two *xantiana* populations at similar elevations, and the Chimney Peak population of *parviflora* flowered more than a week earlier than the Greenhorn Mountain Road population of *xantiana*, which was 200 m lower in elevation. The DIs of the sites where these taxa were found flowering, however, did not reveal strong environmental differences between them (fig. 1).

Physiological Differences between Sister Taxa

Where sister taxa differed significantly in mean gas exchange rates (with variation in T_{leaf} and leaf position statistically controlled for), the selfing taxon always exhibited higher rates than its outcrossing sister taxon (fig. 2). Among populations sampled before flowering, *exilis* and *parviflora* exhibited significantly higher rates of photosynthesis and conductance than *unguiculata* and *xantiana*, respectively. In addition, *parviflora* had higher rates of transpiration than *xantiana*. Among populations sampled during flowering, *exilis* did not differ from *unguiculata* with respect to any trait, but *parviflora* exceeded *xantiana* in its mean rates of photosynthesis and transpiration.

Differences between sister taxa in WUE_i (with T_{leaf} and leaf position controlled for) were detected only before flowering, and the relative difference between autogamous and pollinator-dependent taxa depended on the sister pair. *Exilis* exhibited higher WUE_i than *unguiculata*, while *parviflora* had lower WUE_i than *xantiana* (fig. 2D).

Temporal Changes in Air Temperature and Raw Physiological Rates

Within each taxon, mean air temperatures of individuals sampled before flowering were significantly cooler than those recorded during flowering (fig. 3). Leaf temperatures were highly correlated with air temperature; in *exilis*: $r = 0.96$, $n = 258$; in *unguiculata*: $r = 0.97$, $n = 335$; in *parviflora*: $r = 0.98$, $n = 310$; in *xantiana*: $r = 0.99$, $n = 383$).

Mean raw physiological rates and WUE_i usually differed between preflowering and flowering life stages as well, but not always in the same direction (fig. 4). In *exilis*, the change in mean air temperature between preflowering and flowering sampling dates was significant but modest (an increase of $2.4°C$); mean photosynthetic and transpiration rates also increased ($P < 0.06$ for transpiration) between preflowering and flowering samples, but WUE_i did not change significantly. The other three taxa experienced much larger in-
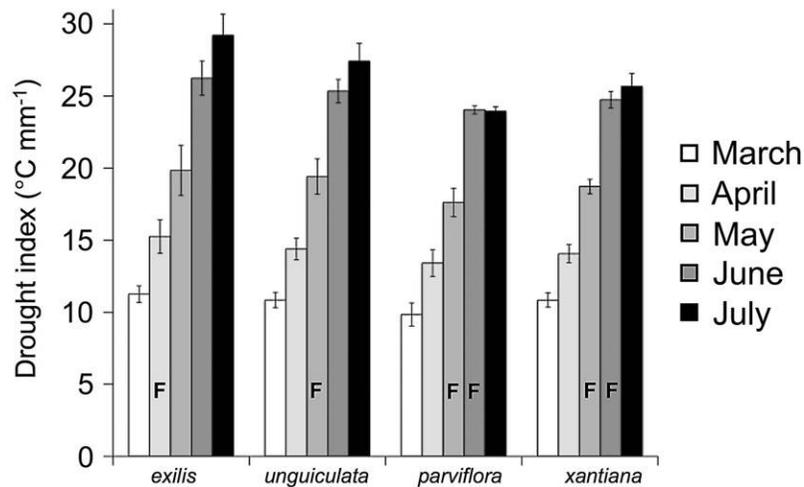
Fig. 1 Mean (± SE) monthly environmental conditions at sites occupied by one or more *Clarkia* taxa (see table 2 for sensor locations and presence of taxa). Mean monthly drought indices were estimated by monthly mean temperatures (°C) minus one-third of monthly total precipitation at sites occupied by each taxon. Mean temperatures are based on records obtained at 15-min intervals. For each taxon, the mean monthly drought indices for the sampled locations are shown, with the months during which most of its flowering occurs indicated by *F*.

creases in air and leaf temperatures between preflowering and flowering stages (mean changes ranged from 7.8° to 11.9°C). In these taxa, mean air temperature exceeded 26°C during flowering, and the mean photosynthetic rates measured at this time were significantly lower than those before flowering.

The temporal reduction in photosynthetic rates observed in *unguiculata*, *parviflora*, and *xantiana* was not always accompanied by a reduction in transpiration, as would be expected if reduced photosynthetic rates were due entirely to stomatal closure. *Unguiculata* exhibited a significant decline in mean transpiration rate as mean air temperature increased from 20.6° to 29.0°C, but *parviflora* and *xantiana* maintained stable transpiration rates even as the mean temperature increased, respectively, from 18.5° to 26.3°C and from 15.5° to 27.4°C.

In the three taxa that experienced temperatures greater than 26°C when sampled during flowering, mean WUE_i declined significantly between the preflowering and flowering sampling dates (fig. 4). This decline in WUE_i was proximally due to the fact that photosynthetic rates declined more drastically over time than did transpiration rates.

Temporal Changes in Physiological Rates, Controlling for Variation in Temperature and Leaf Position

Independent of variation in T_{leaf} and leaf node number, individuals of *exilis* increased their photosynthetic rates over time (fig. 5); *unguiculata* exhibited the opposite trend. In addition, *exilis* showed no significant effect of life stage on WUE_i, while *unguiculata* plants exhibited a significant temporal increase in WUE_i. By contrast, gas exchange rates in *parviflora* did not change significantly over time, while *xantiana* showed a temporal reduction in mean photosynthetic rate similar to that of *unguiculata*.

Relationship between Photosynthetic Rate and Lifetime Fruit Production

For all taxa, high photosynthetic rates were associated with higher lifetime reproductive output, but the strength of the relationship sometimes differed between life stages (table 5). In *unguiculata*, individuals with high photosynthetic rates measured before flowering produced more fruits than those with low rates, but there was no such effect among plants sampled during flowering. In *exilis*, the positive relationship between fruit production and photosynthetic rate was observed both before and during flowering. In *xantiana*, high photosynthetic rates were associated with high fruit production among individuals sampled during flowering but not among those sampled before flowering; *parviflora* showed the opposite pattern. Although the positive contribution of higher photosynthetic rates to this estimate of lifetime fitness was consistent across taxa, variation in photosynthetic rates accounted for no more than 21% of the variation in fruit production (R^2 values ranged from 0.03 to 0.21; table 5).

Differences between Sister Taxa in Stem Biomass and Lifetime Fruit Production

In both pairs of sister taxa, the predominantly outcrossing taxa consisted of consistently larger plants than autogamous selfers, but the latter produced significantly more fruits per plant (fig. 6). Plants of *unguiculata* had a moderately higher mean stem biomass than those of *exilis* ($F_{1,121} = 3.17$, $P < 0.07$; *unguiculata*: $n = 73$; *exilis*: $n = 49$), but *exilis* significantly exceeded *unguiculata* in lifetime fruit production ($F_{1,113} = 12.94$, $P < 0.0005$; *unguiculata*: $n = 73$; *exilis*: $n = 49$). Similarly, *xantiana* plants were significantly larger than *parviflora* plants ($F_{1,204} = 7.23$, $P < 0.0078$; *xantiana*: $n = 122$; *parviflora*: $n = 83$), while *parviflora* had significantly higher fruit production than *xantiana* ($F_{1,203} = 5.21$, $P = 0.0234$; *xantiana*: $n = 122$; *parviflora*: $n = 83$).
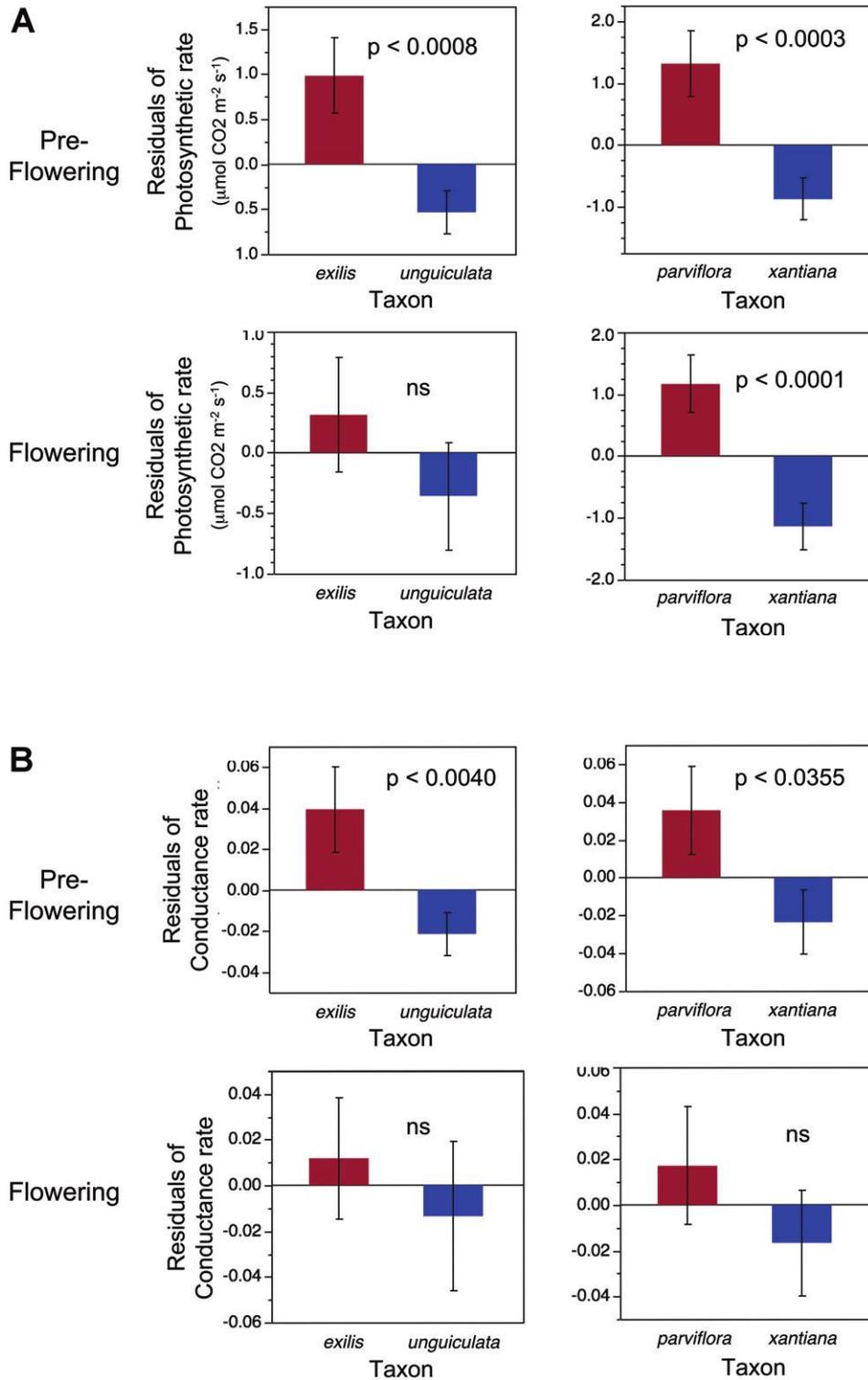
Fig. 2 Mean residuals (± SE) of physiological rates of each taxon; residuals were calculated after regression of each physiological trait on leaf temperature and node number. Conductance (*B*) and transpiration rates (*C*) were measured in mol H_2O m^{-2} s^{-1}. Within each pair of sister taxa, populations sampled before and during flowering were analyzed separately. Within each sister pair and life stage (preflowering vs. flowering), significant differences between sister taxa are indicated by $P < 0.05$.
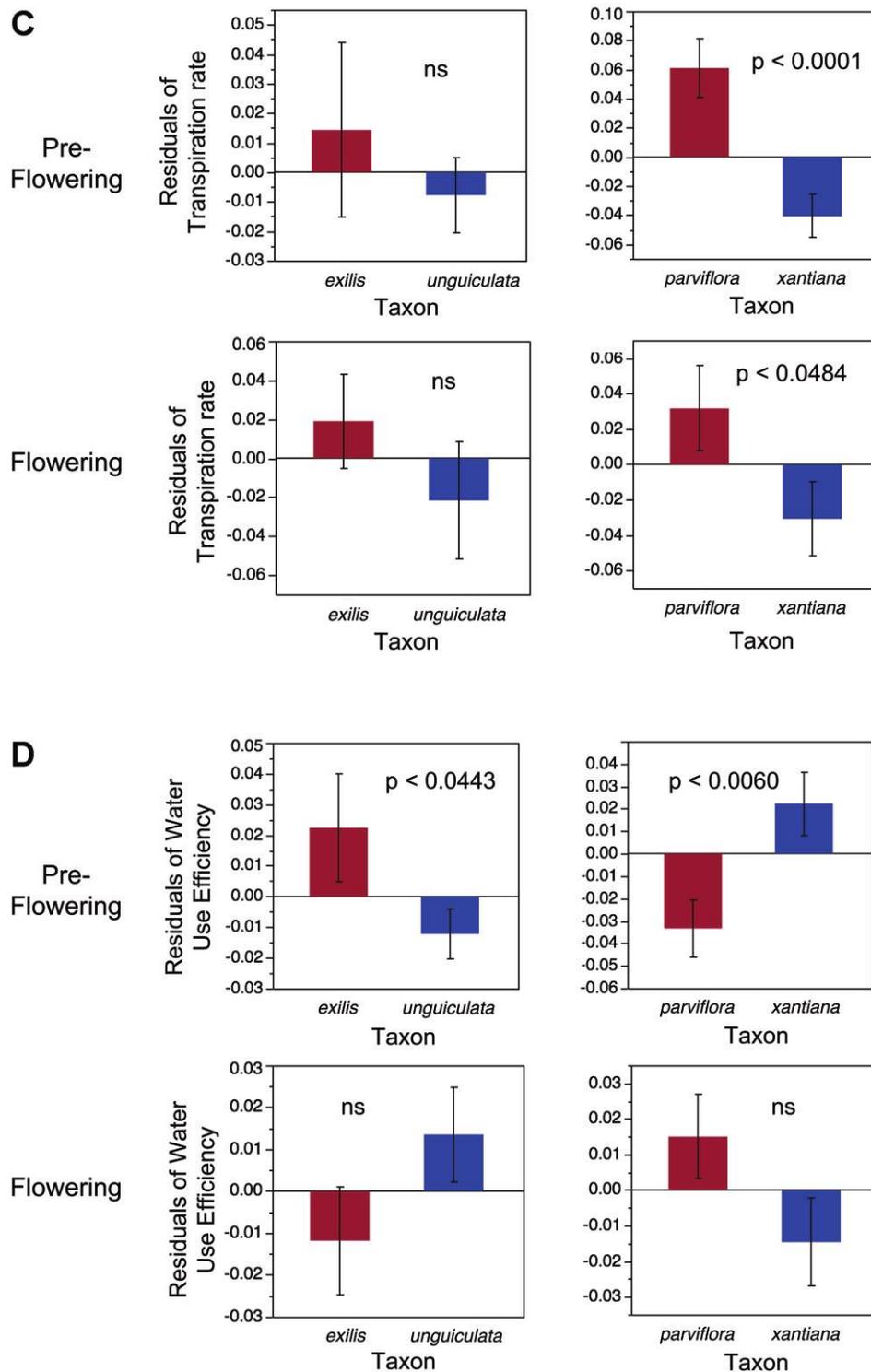
Fig. 2 (*Continued*)

Discussion

The highly autogamous taxa examined here typically have higher gas exchange rates than their outcrossing sister taxa (particularly when T_{leaf} and position are controlled

for). One explanation for this pattern is that natural selection favors higher gas exchange rates in autogamous taxa because of the advantages for plant growth and reproduction (table 5), while selection favoring rapid photosynthesis in the later-flowering outcrossers is weaker because of the
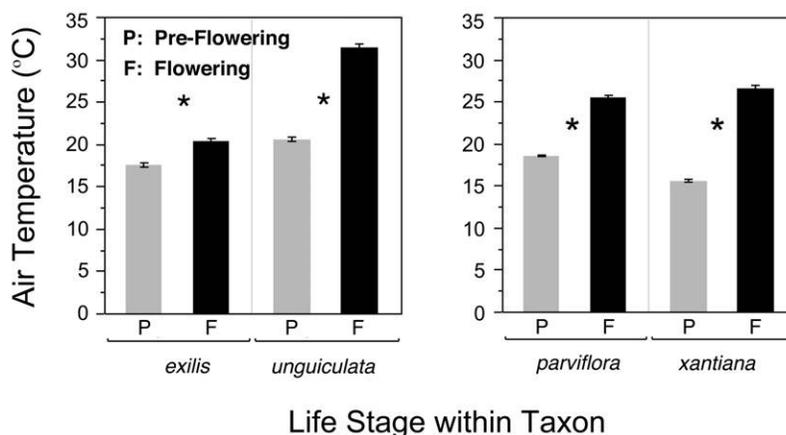
Fig. 3 Taxon means (± SE) for air temperature measured as physiological rates were recorded on each sampled leaf before and during flowering. Within each taxon, data for all populations were pooled. Significant differences in mean air temperature between preflowering and flowering sample dates were detected by one-way ANOVA within each taxon.

transpirational cost of photosynthesis as water stress increases. An alternative explanation is that gas exchange rates are phenotypically plastic and that the earlier flowering of the autogamous taxa permits higher gas exchange rates because of the particular temperature regime and soil moisture that they experience.

While several studies have found genetically based variation in gas exchange traits within taxa, indicating that they may well evolve in response to natural selection, these traits have also been shown to respond plastically to environmental conditions. For example, Caruso et al. (2005) detected significant heritable genetic variation in photosynthetic rate in *Lobelia siphilitica*, while Maherali et al. (2009, 2010) found that recombinant inbred lines of *Avena barbata* exhibited plastic physiological responses to soil moisture conditions before and during reproduction (see Heschel et al. 2004; Heschel and Riginos 2005; and Caruso et al. 2006 for examples of physiological plasticity in other taxa).

Physiological Differences between Sister Taxa

During the life stages where significant differences in photosynthetic and transpiration rates were observed between sister taxa, the selfers had higher gas exchange rates than their outcrossing sister taxa (fig. 2). These results support our hypothesis that selfing taxa have adopted a drought-escape physiological strategy. One caveat tempers this interpretation: the physiological rates were estimated per unit leaf area, which may not reflect differences at the whole-plant level. If selfers have fewer or smaller physiologically active leaves than their outcrossing sister taxa, then their higher photosynthetic rates per square centimeter of leaf tissue would not necessarily enable them to achieve higher individual growth or development rates.

By contrast, our predictions regarding WUE_i—based on assumptions about the adaptive significance of WUE_i (Mooney et al. 1976; Dudley 1996a, 1996b; Heschel and Riginos 2005; Sherrard and Maherali 2006; Donovan et al. 2007; Wu et al. 2010)—were not supported. We expected that water stress would be particularly intense for the large-flowered, late-flowering outcrossers, which experience high DIs in May and June and potentially higher evaporative water loss through their relatively large petals (see Galen et al. 1999; Galen 2000). Accordingly, we predicted that these taxa would exhibit higher WUE_i than their selfing counterparts during flowering. Differences between sister taxa in WUE_i, however, were observed only before flowering, and the relative WUE_i of selfers and outcrossers depended on the sister pair; *xantiana* had higher WUE_i than *parviflora*, but *exilis* had higher WUE_i than *unguiculata*.

The latter result was particularly surprising, given the lower DIs experienced by *exilis* sites relative to where *unguiculata* flowers in May (fig. 1) and the lower T_{leaf}'s recorded in *exilis*. We would emphasize, however, that our measures of WUE were instantaneous (not integrated) measures and may not reflect the values of this parameter observed over a longer time period. Isotope analyses are currently planned to provide integrated measures of WUE for these taxa.

Other recent studies of the adaptive significance of WUE have found a variety of patterns. Dudley (1996a) found that WUE was higher in dry than in wet environments in *Cakile edentula* var. *lacustris*, and Heschel et al. (2002) found that high WUE increased fitness of *Impatiens capensis* in dry field conditions during a late-season drought, but a later experimental study of the same species found selection for lower WUE during early-season drought (Heschel and Riginos 2005). In *A. barbata*, dry conditions favored early flowering, but there was no correlated selection between early flowering and high photosynthetic rates (WUEs were not reported), as would be expected for a drought-escape physiological strategy (Sherrard and Maherali 2006). In contrast, Casper et al. (2005) found survival selection for lower WUE in *Cryptantha flava*, and Donovan et al. (2007) discovered that there was direct selection for low WUE in *Helianthus anomalus* in both mesic and dry habitats and indirect selection for lower WUE in *Helianthus deserticola* in dry environments. Thus, WUE and its adaptive significance may be highly context dependent and not easily predictable from consideration of the
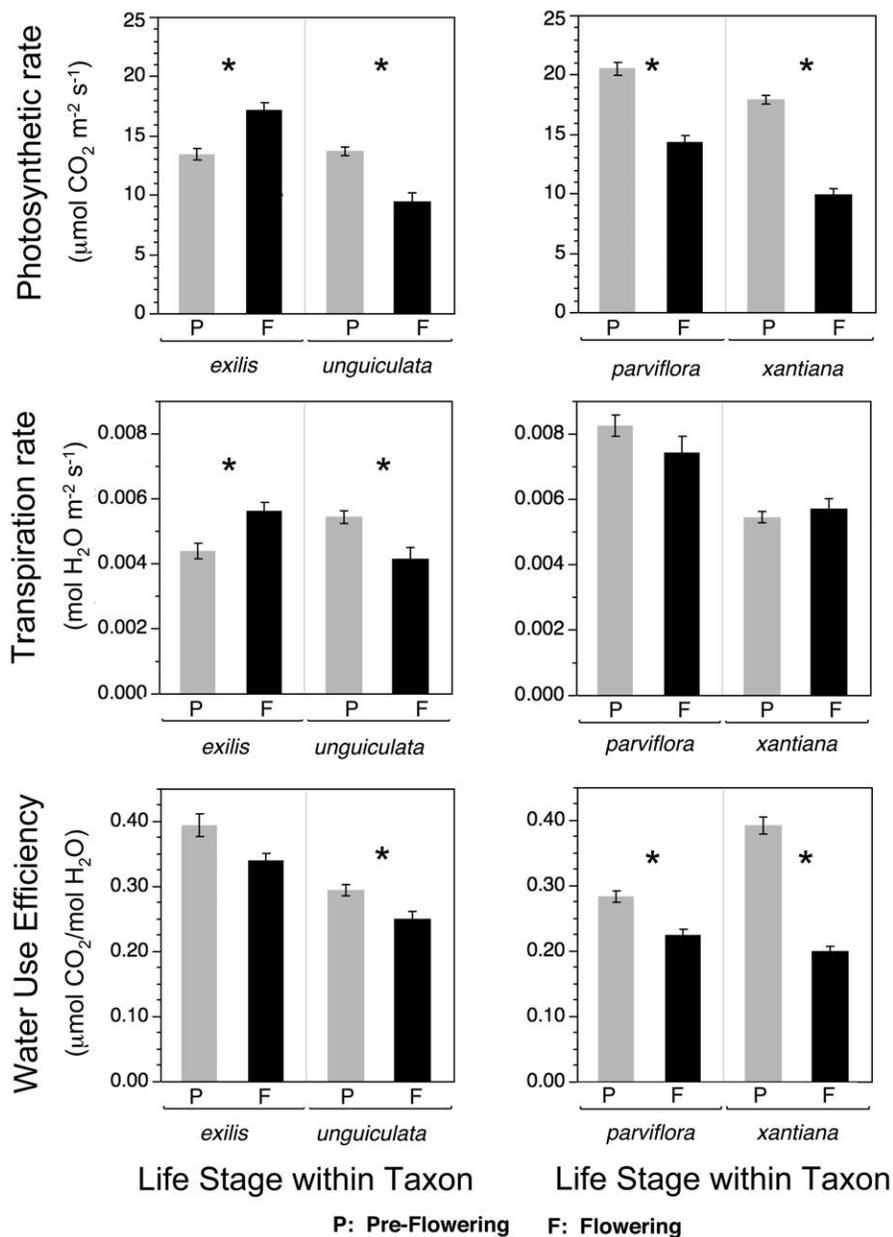
Fig. 4 Taxon means ($\pm$ SE) for the raw values of photosynthetic rate, transpiration rate, and instantaneous water-use efficiency measured on preflowering (*P*) and flowering (*F*) populations. Within each taxon, data for all populations were pooled. Significant differences in mean gas exchange rates between preflowering and flowering sample dates were detected by one-way ANOVA within each taxon, using $\log_{10}$-transformed values of all traits except photosynthetic rates. In all cases, population effects were not significant, so population was excluded from the model. Within taxa, preflowering and flowering population means that are significantly different at the $P < 0.05$ level are indicated by an asterisk.

drought-escape and dehydration-avoidance strategies alone. Other influences may include the precise timing of drought relative to flowering phenology (Heschel and Riginos 2005) and the need to increase nutrient delivery even under drought conditions (Donovan et al. 2007).

Temporal Changes in Raw Physiological Rates

We predicted that the outcrossing taxa would exhibit larger temporal changes in absolute physiological rates because of the reduced water availability and greatly increased temperatures to which they would be exposed during flowering (figs. 1, 3). Consistent with this prediction, the raw photosynthetic rates of *unguiculata* and *xantiana* were significantly lower during flowering than before flowering (fig. 4). However, high temperatures during flowering were not restricted to these late-flowering taxa: *parviflora* populations sampled in 2008 also experienced very hot temperatures during flowering and showed substantial temporal reductions in rates of photosynthesis and transpiration (figs. 3, 4). Thus, although
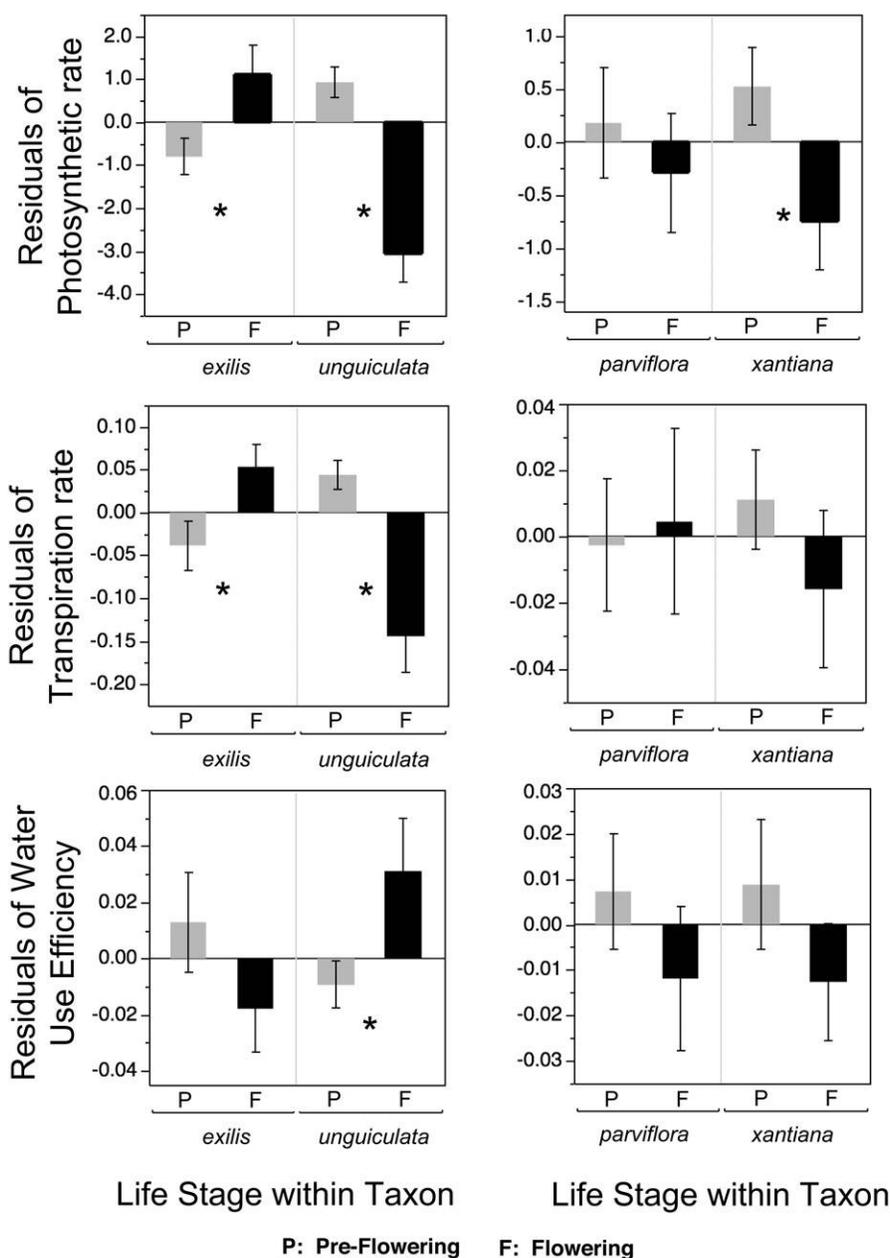
Fig. 5 Mean residuals ($\pm$ SE) of physiological rates of each taxon recorded in populations sampled before (*P*) and during (*F*) flowering. Each taxon was analyzed separately to detect significant temporal changes in physiological rates between the preflowering and flowering stages. Within each taxon, significant differences between life stages in the mean value of physiological traits are indicated by an asterisk when $P < 0.05$.

parviflora flowers earlier than its sister taxon (*xantiana*), it may experience similar levels of water stress. Consequently, apart from the very early–flowering, autogamous *exilis*, all taxa appear to reduce photosynthetic rates over the season in order to minimize transpirational losses, regardless of their mating system.

If the temporal reduction in the photosynthetic rates of *unguiculata*, *xantiana*, and *parviflora* were due entirely to stomatal closure, we would expect to see parallel declines in transpiration rates, but such a decline was seen only in *unguiculata*. In addition, counter to our prediction, no taxon

exhibited a significant increase in the raw value of WUE_i as drought and temperature increased through the season (fig. 4). Indeed, three of the taxa (all but *exilis*) showed significant declines in WUE_i as the temporal declines in photosynthetic rate exceeded the declines in transpiration rate. It is possible that higher transpiration rates are maintained to allow evaporative cooling and to prevent excessive heat stress (Nobel 1991; Lambers et al. 2008). Other processes, such as decreased RuBP regeneration, increased photoprotective energy dissipation, or increases in photorespiration or the Mehler reaction, may also account for the independence between photo-

Table 5

Summary of Regressions of Lifetime Fruit Production versus the Residual of Photosynthetic Rate on Leaf Temperature and Node Number of the Sampled Leaf

Species/subspecies, life stage	n	Regression equation	R^2	P
Clarkia unguiculata:				
Early	150	$y = .05x + .006x^2 + .89$	.14	<.0001
Late	99	$y = .02x + 1.08$	.03	ns
Clarkia exilis:				
Early	96	$y = .05x + .80$	.21	<.0001
Late	128	$y = .04x + 1.27$	.19	<.0001
Clarkia xantiana ssp. *xantiana*:				
Early	108	$y = -.018x + .76$	.03	ns
Late	122	$y = .02x + 1.18$	.06	<.0056
Clarkia xantiana ssp. *parviflora*:				
Early	133	$y = .03x + 1.03$	.17	<.0001
Late	165	$y = .006x + 1.20$	.01	ns

Note.   Regression equations (linear or polynomial, depending on which provided the best fit) are shown; $y = \log_{10}(\text{fruitnumber} + 1)$; x = residuals of photosynthetic rate. Sample size (n) refers to the total number of individuals recorded in each species and life stage; these sample sizes differ from those obtained for physiological rates because not all individuals remained intact when collected to record lifetime fruit production. ns = not significant.

synthetic and transpiration rates (Ort et al. 1994; Cornic and Massacci 1996; Asada 1999; Tezara et al. 1999; Chaves et al. 2002, 2003; Flexas and Medrano 2002; Grassi et al. 2009).

Temporal Changes in Physiological Rates, Controlling for Variation in T_{leaf} and Node Number

Controlling statistically for physiological variation associated with T_{leaf} and node position revealed differences be-

tween sister taxa that were similar to those shown by the raw values (figs. 4, 5). The analyses of the residualized values were, however, statistically more conservative. There were two striking differences between the two analyses. First, although *unguiculata* exhibited a significant temporal decline in raw WUE_i (fig. 4), its residualized WUE_i increased over time (fig. 5). Second, although the raw photosynthetic rates and WUE_i of both *parviflora* and *xantiana* declined over time, the residualized values of these traits did not change
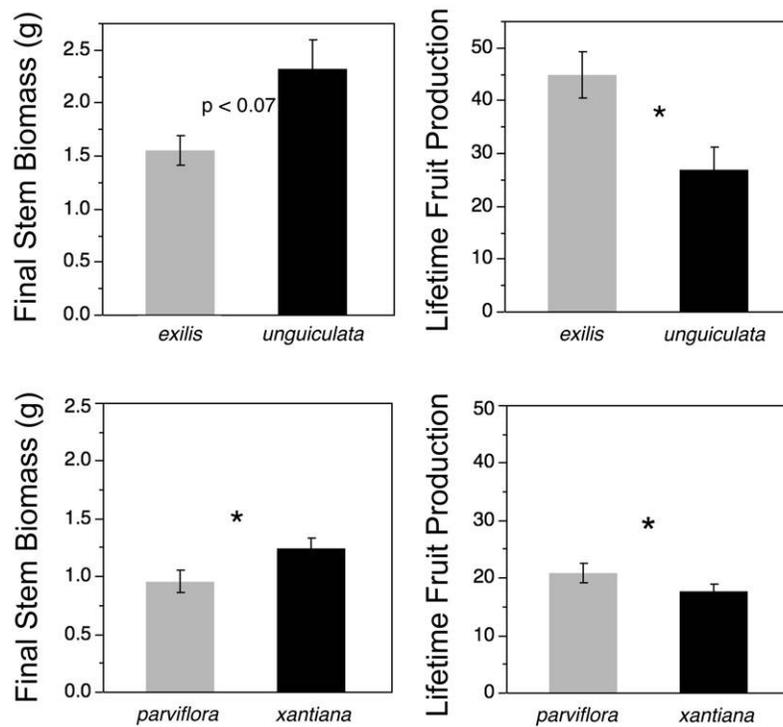

Fig. 6 Mean (± SE) aboveground stem biomass and lifetime fruit production of plants sampled from flowering populations of each taxon. Significant differences between taxon means are indicated by an asterisk.

significantly (with the exception of *xantiana*'s temporal decline in photosynthesis; fig. 4). The differences between the raw and residualized patterns are due to the fact that only the latter statistically take into account correlations between temperature, development stage, and time. Further experimental work is necessary to isolate the independent effects of each of these factors on the physiological traits themselves.

Relationship between Photosynthetic Rate and Lifetime Fruit Production

Within each taxon, the rate of photosynthesis was positively correlated with lifetime fruit production before and/or during flowering (table 5). This suggests that the higher rates of photosynthesis exhibited by the autogamous taxa relative to their pollinator-dependent sister taxa (fig. 2) may provide an intrinsic fitness advantage. Consistent with this idea, we found that selfers achieved higher fruit production than their outcrossing sister taxa, despite their lower individual biomass. The lower lifetime fruit production of the pollinator-dependent taxa could be due to pollen limitation, although seed production per individual fruit in *unguiculata* and *xantiana* does not appear to be pollen limited in their natural habitats (A. A. Hove, L. S. Dudley, and S. J. Mazer, unpublished data, 2008–2009).

Other researchers have also observed positive effects among individuals or genotypes of photosynthetic rate per unit leaf area on components of fitness. Tonsor and Goodnight (1997) detected a positive relationship between photosynthetic rate and reproductive dry weight in *Plantago lanceolata*, while Geber and Dawson (1990) found that, under relatively dry conditions, genotypes of *Polygonum arenastrum* that flower at relatively basal nodes have higher photosynthetic rates and higher fitness than those that flower at higher nodes. In a phenotypic-selection analysis conducted on *C. edentula* var. *lacustris*, Dudley (1996*a*) observed that selection favored higher WUE_i under experimentally dry conditions but not under relatively wet conditions. In a series of experiments using photosynthetically compromised mutants versus wild-type genotypes of *Amaranthus hybridus*, Arntz et al. (1998, 2000*a*, 2000*b*) found that photosynthetic rates had significant (and usually positive) effects on reproductive performance but that the magnitude of the effect was context specific. By contrast, Sherrard and Maherali (2006) found that in the highly selfing *A. barbata*, the linear selection differential (which estimates both direct and indirect selection on a trait) on photosynthetic rate was neutral under dry experimental conditions and positive under well-watered conditions.

Differences between Sister Taxa in Stem Biomass and Lifetime Fruit Production

In both pairs of sister taxa, the pollinator-dependent taxa produced greater aboveground stem biomass than their selfing counterparts (excluding leaves, fruits, and flowers), but the selfers produced as many or significantly more fruits in their lifetime. One interpretation is that the higher photosynthetic rates observed in the selfers (fig. 2) contribute directly to this higher reproductive output. Alternatively, if outcross-

ers incur a greater metabolic cost by producing larger and longer-lived flowers than their autogamous sister taxa, then the latter may be able to convert a higher proportion of their carbon gain into fruit production.

Causes of Mating-System Shifts in Clarkia

The direct benefits of reproductive assurance in the absence of pollinators are intuitively obvious. Indeed, Moeller (2006) found that selfing provides reproductive assurance in *parviflora* populations. Previous research has also shown that *xantiana* and *parviflora* populations differ in both the composition of pollinator species and the quality of pollination service that plants receive (Fausto et al. 2001; Moeller 2005).

In this study, we provide provisional evidence suggesting that the selfing rate may be under indirect selection as well. Early-flowering, autogamous taxa exhibited higher gas exchange rates and a more compressed life cycle, consistent with a drought-escape strategy. Moreover, across taxa, these physiological and life-history traits are associated with the production of small and rapidly developing flowers with low herkogamy and dichogamy (table 1). If these cross-taxon associations mirror genetic correlations within taxa, then direct selection on physiological and life-history traits may result in the correlated evolution of floral development and mating system in the absence of any direct advantages of selfing, or it may reinforce the evolution of autogamy when it is directly advantageous, for reasons of reproductive assurance.

The results presented here are only a first step toward determining whether sister taxa with contrasting mating systems exhibit genetically based differences in physiological performance. Reciprocal-transplant and/or common-garden experiments are valuable complementary approaches for making this determination, although they have the disadvantage of removing genotypes from their natural growing conditions. Moreover, determining whether the observed differences are likely to be due to the adaptive outcome of natural selection will require estimates of the strength and direction of phenotypic selection on each physiological trait, in each taxon, under field conditions (Ackerly et al. 2000; Arntz and Delph 2001). These additional studies are currently underway.

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