Applications in Plant Sciences 2016 4(2): 1500107

Primer Note

Microsatellites for *Oenothera gayleana* and *O. hartwegii* subsp. *filifolia* (Onagraceae), and their utility in section *Calylophus*[1]

Emily M. Lewis[2,3], Jeremie B. Fant[2], Michael J. Moore[4], Amy P. Hastings[5], Erica L. Larson[5,6], Anurag A. Agrawal[5], and Krissa A. Skogen[2,7]

[2]Plant Biology and Conservation, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois 60022 USA; [3]Program in Plant Biology and Conservation, Northwestern University, 2205 Tech Drive, Evanston, Illinois 60208 USA; [4]Department of Biology, Oberlin College, 119 Woodland Street, Oberlin, Ohio 44074 USA; [5]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853; and [6]Division of Biological Sciences, University of Montana, 32 Campus Drive HS104, Missoula, Montana 59812

- *Premise of the study:* Eleven nuclear and four plastid microsatellite markers were screened for two gypsum endemic species, *Oenothera gayleana* and *O. hartwegii* subsp. *filifolia*, and tested for cross-amplification in the remaining 11 taxa within *Oenothera* sect. *Calylophus* (Onagraceae).
- *Methods and Results:* Microsatellite markers were tested in two to three populations spanning the ranges of both *O. gayleana* and *O. hartwegii* subsp. *filifolia*. The nuclear microsatellite loci consisted of both di- and trinucleotide repeats with one to 17 alleles per population. Several loci showed significant deviation from Hardy–Weinberg equilibrium, which may be evidence of chromosomal rings. The plastid microsatellite markers identified one to seven haplotypes per population. The transferability of these markers was confirmed in all 11 taxa within *Oenothera* sect. *Calylophus*.
- *Conclusions:* The microsatellite loci characterized here are the first developed and tested in *Oenothera* sect. *Calylophus*. These markers will be used to assess whether pollinator foraging distance influences population genetic parameters in predictable ways.

Key words: gypsum endemism; microsatellites; *Oenothera* sect. *Calylophus*; Onagraceae; population genetics.

The genus *Oenothera* L. (Onagraceae) has diversified across diverse habitats of North America with conservative shifts in pollinators (primarily between bees and hawkmoths; Raven, 1979) and more dramatic shifts in life history traits (Evans et al., 2009). *Oenothera* sect. *Calylophus* (Spach) Torr. & A. Gray (Onagraceae) consists of seven recognized species (13 taxa) divided into subsections *Calylophus* (Spach) W. L. Wagner & Hoch (*O. capillifolia* Scheele, *O. gayleana* B. L. Turner & M. J. Moore, and *O. serrulata* Nutt.) and *Salpingia* (Torr. & A. Gray) W. L. Wagner & Hoch (*O. hartwegii* Benth., *O. lavandulifolia* Torr. & A. Gray, *O. toumeyi* (Small) Tidestr., and *O. tubicula* A. Gray) (Wagner et al., 2007; Turner and Moore, 2014). Ring chromosomes have been documented in all taxa in sect. *Calylophus* (Towner, 1977), with only *O. serrulata* exhibiting permanent translocation heterozygosity (Johnson et al., 2014).

Oenothera gayleana and *O. hartwegii* subsp. *filifolia* (Eastw.) W. L. Wagner & Hoch are gypsum endemics that often co-occur in eastern New Mexico and western Texas, easily distinguished by floral characteristics associated with bee pollination and hawkmoth pollination, respectively (Towner, 1977; Turner and Moore, 2014). Because bees forage close to nesting sites (Greenleaf et al., 2007) while hawkmoths can travel great distances (Stockhouse, 1973; Alarcón et al., 2008), differentiation between populations is expected to differ between these two plant species (Finger et al., 2014). Here, we characterize 11 nuclear and four plastid microsatellite loci to be used to contrast pollen and seed dispersal patterns in *O. gayleana* and *O. hartwegii* subsp. *filifolia*. We also describe the transferability of these markers to all 11 other taxa in sect. *Calylophus*.

METHODS AND RESULTS

We tested a combination of nuclear and plastid microsatellite loci. We screened 36 unpublished nuclear microsatellite markers that were originally developed for *O. biennis* L., using the microsatellite library prepared by Larson et al. (2008) for studies of genotypic identification and herbivory (Agrawal et al., 2012). In addition, the plastid genome of *O. elata* Kunth subsp. *hookeri* (Torr. & A. Gray) W. Dietr. & W. L. Wagner (GenBank accession no. AJ271079; Hupfer et al., 2000) was screened for large strings of single nucleotide repeats. The plastid primers were designed for 12 microsatellite regions using the following settings in Primer3: optimum primer size 20 bp, melting temperature 60°C, and product size range of 100–300 bp (Untergasser et al., 2012).

[1]Manuscript received 18 September 2015; revision accepted 16 October 2015.

The authors thank W. Daichendt, J. Medina, J. M. Keller, H. Flores Olvera, H. Ochoterena, and N. Douglas for laboratory and field assistance; the U.S. Bureau of Land Management and the USDA Forest Service for permits; and the Ecological Genetics Core Facility at Cornell University, specifically S. Bogdanowicz. Funding was provided by the National Science Foundation (DEB 1342873 to K.A.S. and J.B.F., DEB 1054539 to M.J.M., and DEB-1513839 to A.A.A.), the National Geographic Society (M.J.M.), Oberlin College, a Botanical Society of America Graduate Student Research Award (E.M.L.), and the Chicago Botanic Garden.

[7]Author for correspondence: kskogen@chicagobotanic.org

doi:10.3732/apps.1500107

TABLE 1. Characteristics of 11 nuclear and four plastid microsatellite loci tested in *Oenothera gayleana* and *O. hartwegii* subsp. *filifolia*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range[a] (bp)	T_a (°C)	Reaction mix	Fluorescent dye	GenBank accession no.
Nuclear							
Oenbi2diA_C10[b]	F: AGGAGCAAACTGAAGCAGGA	$(GA)_{20}$	181–205 (a)	56	B	D2	KT762972
	R: TTGCAGAACCCAGAATCTGTT		167–179 (b)				
Oenbi2diA_E9	F: TTTGTCAAATCTATTCCCTAACAGC	$(CA)_{11}$	122–191	56	C	D4	KT762971
	R: TGAGAAAACGTTGGCAAGTG						
Oenbi2triA_A1	F: CCACAGCATCACCAAATTCTTACTT	$(TTC)_8$	307–338	52	C	D4	KT762970
	R: GGGGCGCCAGGTATTGTCG						
Oenbi2triA_A5	F: GCTTCGACCCCATTATTCACTACA	$(GCT)_{10}$	173–185	56	A	D2	KT762969
	R: AACAGCAAAGTTGAGAAGGCG						
Oenbi2triA_C6	F: CCGCAAGAGCTAACAACCAAC	$(TGA)_{16}$	82–97 (a)	56	A	D4	KT762968
	R: CCAGCTTTTTCCAGTATTTTCCTA						
Oenbi2triA_D3[c]	F: CAGATTACGGCGAAAGGAGACAAC	$(ATG)_9$	250–271	52	B	D4	KT762967
	R: CGCTCAGGCATCGCATCTC						
Oenbi2triA_E4	F: CTCTACCCTGCAGTTACCAAAAA	$(TCT)_{10}$	232–323	56	A	D4	KT762966
	R: GAGAGGATTCAACGCAGCAACT						
Oenbi2triA_F5[c]	F: GGGACGCGACCTCAGATTC	$(GAT)_8$	185–197	56	A	D3	KT762965
	R: CGCTCAGGCATCGCATCTC						
Oenbi2triA_H1	F: GAGCCGGAATAAACTGATACCACT	$(GCT)_{14}$	185–218	56	B	D3	KT762964
	R: AGCAGAGAAGGCGTCAACCATAAT						
Oenbi2triA_H2	F: TATCTCAGCACTAAAAGCCTCCTC	$(CAT)_{12}$	167–194	56	C	D2	KT762963
	R: GCTTGGGGTTGGTGCTAAT						
Oenbi39tri10	F: AACAAATTTATGCGATTTCGCC	$(CTT)_6$	125–177	52	B	D4	KT900894
	R: CTGGAAGGGGCGACTGAAAC						
Plastid[d]							
OenelCp3	F: CGGGTTTGAGGTTGAATCAT	$(A)_{13} + (A)_{11}$	262–269	52	D	D4	AJ271079[c]
	R: GGGTGGAGTCGCAGAAAAATA						
OenelCp5[b]	F: GATATAGTTCATGGCCTATTAGAGTT	$(CAGAAGATGAGGAAGGAGAAGG)_6$ +	291–438 (a)	52	D	D3	AJ271079
	R: TGATCGAGTGACATTGCTTCTT	$(CAGAAGAGGAAGTAGAAGGGA)_{12}$	319–451 (b)				
OenelCp11	F: GTTATCCGGCACTTGGAAGA	$(A)_9$+$(A)_9(G)_8$	184–198	52	D	D2	AJ271079
	R: GGATTCGCTACAAAAGGGTTG						
OenelCp12	F: CGAACCGTAGACCTTCTCGG	$(A)_{15}$	193–199	52	D	D2	AJ271079
	R: GCACAGGGAGCCATCTCCTTA						

Note: T_a = annealing temperature when run individually.
[a]All values based on 13 taxa listed in Appendix 1.
[b]Amplified two regions.
[c]These primers share a reverse primer sequence and are likely to be amplifying the same region.
[d]In the *O. elata* chloroplast genome, OenelCp3 begins at 86,105 bp, OenelCp5 at 97,669 bp, OenelCp11 at 165,472 bp, and OenelCp12 at 12,302 bp.

Both nuclear and plastid microsatellite regions were initially screened using three randomly selected individuals of three species in sect. *Calylophus*: *O. serrulata* (Crosbyton, TX), *O. lavandulifolia* (Iraan, TX), and *O. hartwegii* subsp. *filifolia* (Caballo Mountains, NM) (Appendix 1). DNA was extracted from field-collected leaf tissue (Appendix 1) using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). For nuclear microsatellite marker amplification, we used a 10-μL reaction containing 5 μL MyTaq DNA polymerase (Bioline, London, United Kingdom), plus 0.125 μL bovine serum albumin (BSA; 0.5 ng/μL), 3.375 μL DNase-free water, 1 μL template DNA, and 0.25 μL of both forward and reverse primers. The forward primers were fluorescently labeled with WellRed D2 (black), D3 (green), or D4 (blue) (Sigma-Proligo, St. Louis, Missouri, USA). PCRs were run at 95°C for 2 min, then 30 cycles of 50 s at 95°C, 30 s at 56°C, and 1 min at 72°C, with a 10-min extension at 72°C. The plastid microsatellite primers were not fluorescently labeled but instead were amplified and labeled in two steps (Schuelke, 2000). The first PCR reaction mix was identical to above except that the forward primer was designed with an M13 sequence (5′-CACGACGTTGTAAAACGAC-3′) added to the 5′ end. The PCR protocol was as follows: 94°C for 3 min, followed by 13 cycles of 40 s at 94°C, 40 s at 52°C, and 2 min at 72°C, with a final extension of 10 min at 72°C. For the second step, an additional 2.5 μL MyTaq DNA polymerase, 2.0 μL DNase-free water, and 0.5 μL of a labeled M13 forward primer (D2, D3, and D4) was added to each reaction to label any PCR products that contained M13 sequences. The second PCR performed another 27 cycles. The resulting PCR products were analyzed and scored using a 400-bp size standard on a CEQ 8000 Genetic Analysis System version 9.0 (Beckman Coulter, Brea, California, USA).

Of the 36 nuclear primer pairs screened, 14 did not amplify (GenBank accession no.: KT762974–KT762987), 10 amplified unreliably (GenBank accession no.: KT62988–KT62997), one was monomorphic (GenBank accession no.: KT762973), and 11 were polymorphic, one of which (Oenbi2diA_C10; Table 1) amplified two regions in *O. hartwegii* subsp. *filifolia*. These 11 polymorphic markers were further characterized using three populations of *O. gayleana* and two populations of *O. hartwegii* subsp. *filifolia* (10–30 individuals per population; Table 2). To test for cross-amplification, they were also tested on three to five individuals from one population of each of the remaining 11 taxa in *Oenothera* sect. *Calylophus* (Tables 3 and 4, Appendix 1).

For the nuclear microsatellites, we report the following parameters for two to three populations of *O. gayleana* and *O. hartwegii* subsp. *filifolia*: sample size (*N*), number of alleles (*A*), number of private alleles (A_p), observed heterozygosity (H_o), expected heterozygosity (H_e), and deviation from Hardy–Weinberg equilibrium (HWE) (Table 2, calculated using GenAlEx; Peakall and Smouse, 2006). Significant deviation from HWE was observed in at least one population for eight primer pairs in *O. gayleana* and in four primer pairs in both populations of *O. hartwegii* subsp. *filifolia* (Table 2). Primer pairs were tested for linkage disequilibrium for each pair of loci within and across all populations using the log likelihood ratio statistic and Fisher's method in GENEPOP (Raymond and Rousset, 1995). No significant linkage disequilibrium ($P < 0.01$) was detected in either species, except two primer pairs (Oenbi2triA_D3 and Oenbi2triA_F5; Table 1) that share a reverse primer sequence and therefore are likely to be amplifying the same region. For each population, the presence of null alleles at each locus was determined using exact tests in MICRO-CHECKER (van Oosterhout et al., 2004). Any potential null alleles detected in MICRO-CHECKER corresponded with a primer pair that showed deviation from HWE (e.g., Oenbi2diA_E9). We suspect that these anomalies may be due to the presence of ring chromosomes, documented throughout sect. *Calylophus* (Towner, 1977), or the small number of samples included.

Of the 12 plastid regions tested, four amplified reliably and were polymorphic in the two focal species (Table 1). One region (OenelCp5) occasionally produced two peaks; this may be due to stutter or because this region is located within the inverted repeat in the plastid genome. The peak pairs were repeatable and consistent across individuals, hence only the largest peak was scored. Across all species, these four primer pairs identified 28 haplotypes, with one to seven haplotypes per population. Most haplotypes were unique to each population with the exception of one shared haplotype between *O. lavandulifolia* and *O. hartwegii* subsp. *maccartii* (Shinners) W. L. Wagner & Hoch and one between two populations of *O. gayleana* (Yeso 62/180 and Fort Sumner; Tables 3 and 4).

CONCLUSIONS

The 11 nuclear and four plastid microsatellite markers were polymorphic and reliable in *O. gayleana* and *O. hartwegii*

TABLE 2. Results of initial primer screening of 11 polymorphic nuclear microsatellite markers developed in *Oenothera gayleana* (three populations) and *O. hartwegii* subsp. *filifolia* (two populations).

	O. gayleana														*O. hartwegii* subsp. *filifolia*									
	Yeso Hills					Yeso 62/180					Fort Sumner				Yeso Hills					Caballo Mountains				
Locus	*N*	*A*	A_p	H_o	H_e	HWE[b]	*N*	*A*	A_p	H_o	H_e	HWE[b]	*N*	*A*	A_p	H_o	H_e	HWE[b]	*N*	*A*	A_p	H_o	H_e	HWE[b]
Oenbi2diA_C10[a]	15	1	1	0	0	ns	8	1	—	0	0	ns	10	1	—	0	0	ns	26	6	2	0.385	0.768	**	25	7	3	0.28	0.678	***
																			29	3	—	0.276	0.276	ns	28	4	2	0.179	0.167	ns
Oenbi2diA_E9	16	3	1	0	0.32	***	10	3	2	0	0.34	***	10	3	1	0.1	0.265	*	26	17	11	0.423	0.875	***	26	7	2	0.385	0.75	***
Oenbi2triA_A1	16	1	1	0	0	ns	9	1	1	0	0	ns	10	3	—	0.1	0.265	*	27	9	2	0.444	0.764	**	26	10	4	0.269	0.715	***
Oenbi2triA_A5	15	3	—	0.333	0.384	ns	10	4	—	0.3	0.415	ns	10	3	—	0.5	0.405	ns	27	4	2	0.259	0.233	ns	24	1	—	0	0	ns
Oenbi2triA_C6	15	2	2	0.625	0.469	ns	9	4	1	0.111	0.636	**	8	3	2	0.25	0.508	ns	28	1	—	0	0	ns	24	1	—	0	0	ns
Oenbi2triA_D3	16	1	—	0	0	ns	10	3	—	0.1	0.185	***	10	2	—	0	0.18	**	29	6	1	0.517	0.56	ns	29	7	1	0.483	0.45	ns
Oenbi2triA_E4	15	3	—	0.067	0.127	***	9	2	1	0	0.198	**	9	4	—	0.222	0.519	***	29	3	—	0.31	0.445	*	26	7	4	0.423	0.49	***
Oenbi2triA_F5	29	3	—	0	0	ns	10	2	—	0	0.18	**	10	1	—	0	0	ns	28	5	1	0.357	0.364	ns	23	4	—	0.261	0.303	ns
Oenbi2triA_H1	16	3	—	0.313	0.648	*	10	3	—	0.3	0.515	ns	10	3	—	0.6	0.54	ns	29	5	1	0.759	0.6308	ns	29	7	3	0.724	0.666	ns
Oenbi2triA_H2	16	2	1	0.188	0.17	ns	10	4	—	0.4	0.415	ns	10	3	—	0.2	0.445	ns	29	11	5	0.724	0.782	ns	29	7	2	0.655	0.665	ns
Oenbi39tri10	15	2	—	0.067	0.064	ns	10	4	—	0.2	0.27	**	10	2	—	0.6	0.42	ns	27	11	—	0.889	0.874	ns	28	11	—	0.893	0.881	ns

Note: — = not applicable; *A* = number of alleles; A_p = number of private alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = departure from Hardy–Weinberg equilibrium; *N* = number of individuals sampled.

[a] Amplified two regions.

[b] Significant departures from HWE are indicated at the following levels: $^*P = 0.05$, $^{**}P = 0.01$, $^{***}P = 0.001$; ns = not significant.

TABLE 3. Results of cross-amplification of nuclear microsatellites in the 11 additional taxa within *Oenothera* sect. *Calylophus*. Results from *O. gayleana* and *O. hartwegii* subsp. *filifolia* are included for comparison.

Subsection	Species	Population	N nuc	Oenbi2diA_C10[a]		Oenbi2diA_E9	Oenbi2triA_A1	Oenbi2triA_A5	Oenbi2triA_C6	Oenbi2triA_D3	Oenbi2triA_E4	Oenbi2triA_F5	Oenbi2triA_H1	Oenbi2triA_H2	Oenbi39tri10
Calylophus	*O. capillifolia* subsp. *berlandieri*	Monahans	8	—	181–195	120–130	320–323	176–185	—	265–268	232–253	191–194	200–209	191–194	131
	O. capillifolia subsp. *capillifolia*	Uvalde	5	—	183	122–130	310–323	176–188	100–115	265–268	314–355	194	200–206	155–188	134–158
	O. gayleana	Yeso Hills	16	—	183	122–153	316	179–185	94–103	268	244–320	194	203–218	191–194	131–171
		Yeso 62/180	10	177	183	122–153	316	176–185	82–94	250–268	244–320	188–194	203–218	182–194	131–177
		Fort Sumner	10	—	183	122–130	313–326	176–185	85–97	265–268	235–323	194	203–218	185–191	131–134
	O. serrulata	Crosbyton	5	—	170–183	120–130	313–323	176–185	85	268	235–320	194–200	188–209	188–191	131
Salpingia	*O. hartwegii* subsp. *fendleri*	Galisteo Dam	5	—	179–191	155–161	274	176	94–97	259–265	244	188–194	197	185–194	155–170
	O. hartwegii subsp. *filifolia*	Yeso Hills	30	169–179	191–205	143–191	307–335	173–182	94	256–271	241–250	185–197	197–206	167–191	152–177
		Caballo Mtn.	30	167–177	185–205	149–181	310–338	176	94	250–271	232–250	188–197	185–215	167–188	152–177
	O. hartwegii subsp. *hartwegii*	Mazapil	5	177–179	189–193	153–171	313–332	176	94	262–265	244–247	188–194	197–218	182–188	149–177
	O. hartwegii subsp. *maccartii*	Zapata	5	177	183–195	137–153	320–332	176	94	262–278	235–250	188–204	197–203	182–188	149–161
	O. hartwegii subsp. *pubescens*	Ranch 7	5	177	185–193	153–173	271–320	176	94	259	244–247	188–194	203–209	182–185	161–168
	O. lavandulifolia	Iraan	5	177	187–193	124–159	292–320	176	94	262–268	241–250	188–197	200–203	179–191	152–180
	O. toumeyi	Pinery Canyon	5	—	183	157–169	304–307	176	94–112	259–274	241–244	185–200	206–212	188	149–152
	O. tubicula subsp. *strigulosa*	La Ascension	5	177	189–197	143–157	313	176–188	94	262–265	244	188–191	197–206	176–206	152–174
	O. tubicula subsp. *tubicula*	Black River Rd.	5	167–183	189	143–189	310–332	176	94–97	250–262	244–247	188	182–209	197	155–171

Note: N nuc = number of individuals tested with nuclear microsatellite markers.
[a]Amplified two regions.

TABLE 4. Results of cross-amplification of plastid microsatellites in the 11 additional taxa within *Oenothera* sect. *Calylophus*. Results from *O. gayleana* and *O. hartwegii* subsp. *filifolia* are included for comparison.

Subsection	Species	Population	N cp	OenelCp3	OenelCp5[a]	OenelCp11	OenelCp12	N cp haplotypes
Calylophus	*O. capillifolia* subsp. *berlandieri*	Monahans	3	266	338–362	345–369	192	195–199	2
	O. capillifolia subsp. *capillifolia*	Uvalde	3	264	338	344	192	196–199	1
	O. gayleana	Yeso Hills	12	263–269	315–380	319–387	184–197	193–199	7
		Yeso 62/180	3	265	380	388	193	196	1
		Fort Sumner	3	265	380–383	390	193	196	2
		Crosbyton	3	265	280	285	191	195	1
	O. serrulata	Galisteo Dam	na	na	na	na	na	na	
Salpingia	*O. hartwegii* subsp. *fendleri*	Yeso Hills	27	263–269	354–438	363–451	195–198	193–195	7
	O. hartwegii subsp. *filifolia*	Caballo Mtn.	10	262–267	291–297	—	—	195	
	O. hartwegii subsp. *hartwegii*	Mazapil	3	262–265	330–392	338	196	192–193	2
	O. hartwegii subsp. *maccartii*	Zapata	3	263	311	317	193	192	1
	O. hartwegii subsp. *pubescens*	Ranch 7	3	267	371	—	196	195	1
	O. lavandulifolia	Iraan	3	263	311	317	195	192	1
	O. toumeyi	Pinery Canyon	3	269	372	382	196	194	1
	O. tubicula subsp. *strigulosa*	La Ascension	3	263	290	296	196	196	1
	O. tubicula subsp. *tubicula*	Black River Rd.	3	264	372–413	382–425	195	195	2

Note: N cp = number of individuals tested with chloroplast microsatellite markers; na = not available.
[a]Amplified two regions.

subsp. *filifolia* and in some populations of the remaining 11 taxa within *Oenothera* sect. *Calylophus.* These markers will be used in future studies of genetic differentiation between populations in the bee-pollinated *O. gayleana* and the hawkmoth-pollinated *O. hartwegii* subsp. *filifolia*. In addition, they will be useful for investigations into gene flow within and among other taxa in sect. *Calylophus* and may help identify populations and species that exhibit translocation heterozygotes in this group.

LITERATURE CITED

AGRAWAL, A. A., A. P. HASTINGS, M. T. JOHNSON, J. L. MARON, AND J. P. SALMINEN. 2012. Insect herbivores drive real-time ecological and evolutionary change in plant populations. *Science* 338: 113–116.

ALARCÓN, R., G. DAVIDOWITZ, AND J. BRONSTEIN. 2008. Nectar usage in a southern Arizona hawkmoth community. *Ecological Entomology* 33: 503–509.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

EVANS, M., S. A. SMITH, R. S. FLYNN, AND M. J. DONOGHUE. 2009. Climate, niche evolution, and diversification of the "Bird-Cage" evening primroses (*Oenothera*, sections *Anogra* and *Kleinia*). *American Naturalist* 173: 225–240.

FINGER, A., C. KAISER-BUNBURY, C. KETTLE, T. VALENTIN, AND J. GHAZOUL. 2014. Genetic connectivity of the moth pollinated tree *Glionnetia sericea* in a highly fragmented habitat. *PLoS ONE* 9: e111111.

GREENLEAF, S. S., N. M. WILLIAMS, R. WINFREE, AND C. KREMEN. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153: 589–596.

HUPFER, H., M. SWIATEK, S. HORNUNG, R. G. HERRMANN, R. M. MAIER, W.-L. CHIU, AND B. SEARS. 2000. Complete nucleotide sequence of the *Oenothera elata* plastid chromosome, representing plastome I of the five distinguishable *Euoenothera* plastomes. *Molecular & General Genetics* 263: 581–585.

JOHNSON, M. T. J., A. R. IVES, J. AHERN, AND J.-P. SALMINEN. 2014. Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist* 203: 267–279.

LARSON, E. L., S. M. BOGDANOWICZ, A. A. AGRAWAL, M. T. J. JOHNSON, AND R. G. HARRISON. 2008. Isolation and characterization of polymorphic microsatellite loci in common evening primrose (*Oenothera biennis*). *Molecular Ecology Resources* 8: 434–436.

PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

RAVEN, P. H. 1979. A survey of reproductive biology in Onagraceae. *New Zealand Journal of Botany* 17: 575–593.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.

STOCKHOUSE, R. E. 1973. Biosystematic studies of *Oenothera* L. subgenus *Pachylophus.* Ph.D. dissertation, Colorado State University, Fort Collins, Colorado, USA.

TOWNER, H. F. 1977. The biosystematics of *Calylophus* (Onagraceae). *Annals of the Missouri Botanical Garden* 64: 48–120.

TURNER, B. L., AND M. J. MOORE. 2014. *Oenothera gayleana* (*Oenothera* sect. *Calylophus*, Onagraceae), a new gypsophile from Texas, New Mexico, and Oklahoma. *Phytologia* 96: 200–206.

UNTERGASSER, A., I. CUTCUTACHE, T. KORESSAAR, J. YE, B. C. FAIRCLOTH, M. REMM, AND S. G. ROZEN. 2012. Primer3—new capabilities and interfaces. *Nucleic Acids Research* 40: e115.

VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. WILLS, AND P. SHIPLEY. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.

WAGNER, W. L., P. C. HOCH, AND P. H. RAVEN. 2007. Revised classification of the Onagraceae. *Systematic Botany Monographs* 83: 1–240.

Appendix 1. Voucher information, mating system, and primary pollinator for all *Oenothera* sect. *Calylophus* taxa used in this study.

Subsection	Species	Population locality	Latitude	Longitude	Voucher collector no.[a]	Mating system[b]	Primary pollinator[c]
Calylophus	*O. capillifolia* Scheele subsp. *berlandieri* (Spach) W. L. Wagner & Hoch	Monahans, TX, USA	31°36′58.2″N	−102°48′29.3″W	*M. J. Moore 757*	SI	B
	O. capillifolia Scheele subsp. *capillifolia*	Uvalde, TX, USA	29°14′45.3″N	−99°47′23.6″W	*M. J. Moore 1040*	SI	B
	O. gayleana B. L. Turner & M. J. Moore	Yeso Hills, NM, USA	32°02′13.9″N	−104°27′18.8″W	*M. J. Moore 2286*	SI	B
		Yeso 62/180, NM, USA	32°02′36.9″N	−104°28′10.3″W	*M. J. Moore 653*	SI	B
		Fort Sumner, NM, USA	34°09′17.7″N	−104°28′51.6″W	*M. J. Moore 669*	SI	B
	O. serrulata Nutt.	Crosbyton, TX, USA	33°40′21.1″N	−101°10′27.5″W	*M. J. Moore 798*	SC	Self
Salpingia	*O. hartwegii* Benth. subsp. *fendleri* (A. Gray) W. L. Wagner & Hoch	Galisteo Dam, NM, USA	35°27′27.7″N	−106°13′08.8″W	*M. J. Moore 928*	SI	HM
	O. hartwegii Benth. subsp. *filifolia* (Eastw.) W. L. Wagner & Hoch	Yeso Hills, NM, USA	32°02′13.9″N	−104°27′18.8″W	*M. J. Moore 2285*	SI	HM
		Caballo Mountains, NM, USA	33°00′23.4″N	−107°09′25.1″W	*M. J. Moore 2260*	SI	HM
	O. hartwegii Benth. subsp. *hartwegii*	Mazapil, Zacatecas, Mexico	24°38′58.2″N	−101°34′36.7″W	*M. J. Moore 1400*	SI	HM
	O. hartwegii Benth. subsp. *maccartii* (Shinners) W. L. Wagner & Hoch	Zapata, TX, USA	26°51′45.0″N	−99°14′48.1″W	*M. J. Moore 997*	SI	HM
	O. hartwegii Benth. subsp. *pubescens* (A. Gray) W. L. Wagner & Hoch	Ranch 7, TX, USA	30°14′51.9″N	−103°33′56.6″W	*M. J. Moore 601*	SI	HM
	O. lavandulifolia Torr. & A. Gray	Iraan, TX, USA	30°52′29.1″N	−102°05′10.2″W	*M. J. Moore 623*	SI	HM
	O. toumeyi (Small) Tidestr.	Pinery Canyon, AZ, USA	31°56′10.2″N	−109°16′53.8″W	*M. J. Moore 857*	SI	HM
	O. tubicula A. Gray subsp. *strigulosa* (Towner) W. L. Wagner & Hoch	La Ascensión, Nuevo León, Mexico	24°18′15.2″N	−99°53′28.3″W	*M. J. Moore 1367*	SI	B
	O. tubicula A. Gray subsp. *tubicula*	Black River Rd., NM, USA	32°14′20.3″N	−104°12′16.4″W	*M. J. Moore 1077*	SI	B

[a] Herbarium vouchers deposited at the U.S. National Herbarium (US).
[b] SC = self-compatible; SI = self-incompatible.
[c] B = bee; HM = hawkmoth; Self = autogamous.