Cross-compatibility in Intraspecific and Interspecific Hybridization within *Lychnis* and Intergeneric Hybridization between *Lychnis* and *Silene*

Li Jiang¹

Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078-6027

Yun-wen Wang

Department of Grassland Science, College of Animal Science and Technology, China Agricultural University, Beijing 100193, The People's Republic of China

Bruce L. Dunn²

Department of Horticulture and Landscape Architecture, Oklahoma State University, 358 Ag Hall, Stillwater, OK 74078-6027

Additional index words. Caryophyllaceae, ornamental breeding, seed morphology, seed set

Abstract. Hybridization at intraspecific, interspecific, and intergeneric levels is a well-known breeding strategy to create variations with desirable traits for ornamental flowers. A total of 161 crossing combinations were made on three taxonomic levels, including 12 intraspecific crossing combinations within *L. chalcidonica* and *L. ×haageana*, 102 interspecific crossing combinations within *Lychnis* spp., and 47 intergeneric crossing combinations between *Lychnis* spp. and *Silene* spp. Intraspecific crosses showed high cross-compatibility, which yielded mature seeds and progeny plants. Most of the interspecific crossings in genus *Lychnis* produced limited seed set and germination percentages; however, *L. cognate*, *L. ×arkwrightii*, and *L. ×haageana* showed high cross-compatibility and might be more closely related than other species in *Lychnis*. As a result of cross-incompatibility, crossing combinations between *Lychnis* spp. and *Silene* spp. produced few flowers that set seeds. Significant differences occurred in seed set between crossing combinations and their reciprocal crosses for interspecific and intergeneric crosses. For these hybrids with immature seeds, embryo rescue techniques with immature seed culture would be the only way to produce seedlings.

The genera *Lychnis* L. and *Silene* L. belong to the family Caryophyllaceae. *Lychnis* species, commonly known as campion or catchfly, are native to the temperate regions of the Northern Hemisphere, from East Asia to Central Asia, Europe, and North and East Africa (Oxelman et al., 2000; Popp et al., 2008). *Lychnis* is characterized by perennial herbs, consisting of ≈30 species with some species being extensively cultivated as landscape ornamentals. *Silene* is the largest genus in Caryophyllaceae, consisting of biennial or perennial herbs with species numbers varying from 500 to 700 (Melzheimer, 1988; Oxelman et al., 2000; Walters, 1989). *Silene* species occur worldwide, although the greatest diversity occurs in the Mediterranean region (Oxelman et al., 2000). The taxonomic boundary between *Lychnis* and *Silene* L. is

often disputed (Desfeux and Lejeune, 1996; Kruckeberg, 1962). Both genera have been merged in cladistic theory (Desfeux and Lejeune, 1996; Negrutiu et al., 2001), yet *Lychnis* as an independent genus is also accepted by researchers beyond taxonomic fields. Distinction between the traditional genera *Lychnis* and *Silene* in morphology is that *Lychnis* usually has five styles and an entire capsule, whereas *Silene* has three styles and a split capsule (Desfeux and Lejeune, 1996).

Lychnis has ornamental merit because most species are perennial, have good drought tolerance, and exhibit high diversity in morphology and ecophysiology. The majority of *Lychnis* and *Silene* species have identical somatic chromosome numbers ($2n = 2x = 24$) (Negrutiu et al., 2001), which suggests the possibility of hybridization for exploring desirable and exotic hybrids for commercial use. Hybridization has been used to test the phylogeny relationship of *Lychnis* and *Silene* (Kruckeberg, 1962; Wilson et al., 1995). A number of cultivars are commercially available and were obtained from intraspecific breeding (Godo et al., 2009;

Mayol and Rossello, 2006). An exceptional example is *Lychnis ×arkwrightii* Heydt, which is a putative hybrid between *Lychnis chalcidonica* L. and *Lychnis ×haageana* Lemoine. The majority of *Lychnis* species are hermaphrodites and are cross-pollinated by insects, which is aided by being protandrous, although self-pollination does occur. Therefore, crossing combinations were made to test the crossability among *Lychnis* and *Silene* species and cultivars.

Materials and Methods

Plant materials and growth conditions.

Seven *Lychnis* species, totaling 20 accessions including commercial cultivars, hybrids, and species, and five *Silene* species were used for hybridizations (Table 1). A total of 161 crossing combinations were made on three taxonomic levels including: 1) 12 intraspecific crossing combinations within *L. chalcidonica* and *L. ×haageana*; 2) 102 interspecific crossing combinations within *Lychnis* spp.; and 3) 47 intergeneric crossing combinations between *Lychnis* spp. and *Silene* spp. Five crosses including reciprocal crosses were attempted for each combination without emasculation, yet the actual crossing numbers were restricted to flower availability. Overall, 49 crosses were made for intraspecific crossing, 623 crosses were made for interspecific crossing, and 290 crosses were made for intergeneric crossing. A total of 962 crosses were made for all combinations. To facilitate the large number of hybridizations, flowers were not emasculated; however, all progeny were evaluated based on flower and plant morphological traits to determine if progeny was the result of selfing. Progeny was removed from the study if they were a result of selfing. Fertilized capsules were collected as the color changed from green to brown. All hybridizations were conducted during May to Oct. 2010 in the Department of Horticulture and Landscape Architecture Research Greenhouses, Oklahoma State University, Stillwater, OK, under natural photoperiods. Temperature was set at 18/21 °C day/night with a photosynthetic photon flux density range of 600 to 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 1200 HR. Seeds from individual capsules were sown in Metro-mix 702 (Sun Gro Horticulture, Bellevue, WA) media placed in 15-cm round pots (Itml, Middlefield, OH) starting July 2010 in the greenhouse.

Data collection. Beginning in July 2010, the number of crosses, number of successful crosses, F_1 seed number, and germination rates were recorded. Phenotypic traits including leaf color, flower color, and size were recorded starting in May 2011. Hybrid flower morphological data of corolla diameter, petal width, and petal length were recorded on five flowers, each of them from an individual plant. Flower and leaf color were recorded using The Royal Horticultural Society Color Charts (1966). All hybrid plants were cultivated in the greenhouse when the color data were taken. The traits were analyzed using

Received for publication 28 Apr. 2014. Accepted for publication 24 July 2014.

¹Former graduate student. Currently a doctoral student, Department of Plant and Environmental Sciences, New Mexico State University.

²To whom reprint requests should be addressed; e-mail bruce.dunn@okstate.edu.

analysis of variance (PROC GLM, Version 9.3; SAS®, Cary, NC), and means were compared using a Duncan test ($P \leq 0.05$, $n = 5$).

Results

Intraspecific crosses. All 12 of the intraspecific combinations within *Lychnis* species set seed (Tables 2 and 3). Among six accessions within *L. chalconica* and 48 pollinated flowers, on average 83.3% set seed and had an average seed number of 52 per capsule. A total of 2094 seeds was collected from intraspecific crosses within *L. chalconica*, and average germination rate was 64.7% (Table 2). Only one intraspecific cross between *L. xhaageana* ‘Molten Lava’ and *L. xhaageana* mixed hybrids was made, of which 24 seeds were collected from the single capsule and 91.7% of them germinated normally (Tables 2 and 3). These results confirmed that interbreeding within species could successfully produce fertile seed and progeny. For *L. chalconica*, *L. chalconica* var. *alba*, and *L. chalconica* ‘White’, differences in percent of flowers that set seeds and seed germination rates within reciprocal crosses occurred (Tables 2 and 3). In particular, for a cross combination between *L. chalconica* ‘White’ and *L. chalconica*, seed germination rate was 84.8% when *L. chalconica* ‘White’ as a maternal parent, much higher than that of 22.0% as a paternal parent. Red flower was dominant in all intraspecific F_1 hybrid plants among *L. chalconica* (red), *L. chalconica* var. *alba*, *L. chalconica* ‘White’, and *L. chalconica* ‘Rauhreif’ (white), or *L. chalconica* ‘Carnea’ (dusty pink) flowers (data not shown).

Interspecific crosses. Compared with the intraspecific crosses, most interspecific crosses in the genus *Lychnis* produced fewer flowers that set seed and had lower seed germination percentages (Tables 4 and 5). However, the percent of flowers that set seed and germinated was relatively high for the crossing pairs between *L. cognata* and *L. xarkwrightii* ‘Vesuvius’, and *L. xhaageana* (*L. xhaageana* mixed hybrids and ‘Molten Lava’, ‘Lumina Orange’, and ‘Lumina Broze leaf Red’), and also between *L. xarkwrightii* ‘Vesuvius’ and *L. xhaageana* mixed hybrids. In the crossing pairs where *L. cognata* was the maternal parent, 57.1% of crosses with *L. xarkwrightii* ‘Vesuvius’ and 80.0% of crosses with *L. xhaageana* mixed hybrids produced seed and seed germination rates were 77.1% and 68.5%, respectively (Table 6). In crosses with *L. xarkwrightii* ‘Vesuvius’ as the maternal parent and *L. cognata* as the pollen parent, seed set and seed germination rates were 93.8% and 69.2%, respectively. Seed set was lower for the crossing pairs with *L. xhaageana* mixed hybrids where *L. cognata* was the pollen parent; however, seed germination rates were similar (Table 6). In the reciprocal crosses between *L. xhaageana* ‘Molten Lava’ and *L. cognata*, and the crosses between *L. xhaageana* ‘Lumina Orange’ or ‘Lumina Bronze Leaf Red’ with *L. cognata*, 100.0% of crosses produced seeds, and seed germination rates ranged from 53.2% to 87.3% (Table 6). For *L. xarkwrightii* ‘Vesuvius’ $\times$ *L. xhaageana* mixed hybrids, 40.0% of pollinated flowers set seed and 60.7% of the seeds germinated. Meanwhile, the reciprocal crosses between the two accessions yielded 87.5% seed setting flowers and 68.2% seed germination (Table 6). The results demonstrated that no interbreeding barrier exists within the three species including *L. cognata*, *L. xarkwrightii*, and *L. xhaageana* out of the seven species we tested in genus *Lychnis*. They are likely more closely related to each other than the other species.

For the other 403 interspecific crossing pairs in *Lychnis*, except for *L. miqueliana* $\times$ *L. xarkwrightii* ‘Orange Genome’, all of the interspecific crossing pairs produced seeds (Tables 4 and 5). Considering the average

Table 1. *Lychnis* and *Silene* species and cultivars used in hybridization and their seed sources.

Species and cultivars	Seed sources
<i>Lychnis</i>	
<i>L. chalconica</i>	J.L. Hudson, Seedsman, CA
<i>L. chalconica</i> var. <i>alba</i>	Chiltern Seeds, Wallingford, UK
<i>L. chalconica</i> ‘White’	Hardyplants.com, MN
<i>L. chalconica</i> ‘Rauhreif’	Hardyplants.com, MN
<i>L. chalconica</i> ‘Carnea’	Hardyplants.com, MN
<i>L. chalconica</i> ‘Burning Love’	Hardyplants.com, MN
<i>L. cognata</i> Maxim.	B & T World Seeds, Pagnignan, France
<i>L. miqueliana</i> Rohrb.	Arrowhead Alpines—Bob & Brigitta Stewart, MI
<i>L. wilfordii</i> (Regel) Maxim.	Arrowhead Alpines—Bob & Brigitta Stewart, MI
<i>L. flos-cuculi</i> L.	J.L. Hudson, Seedsman, CA
<i>L. flos-cuculi</i> ‘White Robin’	Hardyplants.com, MN
<i>L. xhaageana</i> mixed hybrids	Chiltern Seeds, Wallingford, UK
<i>L. xhaageana</i> ‘Molten Lava’	Seeds-by-Size, Hemel Hempstead, UK
<i>L. xhaageana</i> ‘Lumina Salmon’	Hardyplants.com, MN
<i>L. xhaageana</i> ‘Lumina Orange’	Hardyplants.com, MN
<i>L. xhaageana</i> ‘Lumina Bronze Leaf Red’	Hardyplants.com, MN
<i>L. xarkwrightii</i>	B & T World Seeds, Pagnignan, France
<i>L. xarkwrightii</i> ‘Vesuvius’	J.L. Hudson, Seedsman, CA
<i>L. xarkwrightii</i> ‘Orange Genome’	Hardyplants.com, MN
<i>Silene</i>	
<i>S. armeria</i> L.	B & T World Seeds, Pagnignan, France
<i>S. pendula</i> L.	B & T World Seeds, Pagnignan, France
<i>S. plankii</i> C.L. Hitchc. & Maguire	Alpains, CO
<i>S. acaulis</i> ssp. <i>Acaulescens</i> (F.N. Williams) C.L. Hitchc. & Maguire	B & T World Seeds, Pagnignan, France
<i>S. delavayi</i> Franch.	B & T World Seeds, Pagnignan, France

Table 2. Flower pollination, seed set, and germination for intraspecific crosses as seed parent within *Lychnis*.

Seed parent	No. of cultivars mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalconica</i>	3	16	93.8	52	786	55.7
<i>L. chalconica</i> ‘Rauhreif’	2	5	100.0	66	329	38.9
<i>L. chalconica</i> ‘White’	3	16	81.3	53	686	85.0
<i>L. chalconica</i> var. <i>alba</i>	3	11	63.6	42	293	70.3
Total for <i>L. chalconica</i>	6	48	83.3	52	2094	64.7
<i>L. xhaageana</i> ‘Molten Lava’	1	1	100.0	24	24	91.7

Table 3. Flower pollination, seed set, and germination for intraspecific crosses as pollen parent within *Lychnis*.

Pollen parent	No. of cultivars mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalconica</i>	2	14	78.6	56	614	82.6
<i>L. chalconica</i> ‘White’	1	5	100.0	62	309	22.0
<i>L. chalconica</i> var. <i>alba</i>	1	5	80.0	41	162	77.8
<i>L. chalconica</i> ‘Burning Love’	3	10	70.0	58	405	51.9
<i>L. chalconica</i> ‘Carnea’	4	14	92.9	46	604	73.5
<i>L. xhaageana</i> mixed hybrids	1	1	100.0	24	24	91.7

Table 4. Flower pollination, seed set, and germination for interspecific crosses excluding crosses in Table 6 as the seed parent within *Lychnis*.

Seed parent	No. of species mating	No. of cultivars mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalconica</i>	4	6	49	40.8	43	857	0.0
<i>L. chalconica</i> 'Burning Love'	2	2	10	20.0	25	50	0.0
<i>L. chalconica</i> 'Carnea'	3	3	18	50.0	32	288	0.0
<i>L. chalconica</i> 'Rauhreif'	4	4	14	28.6	12	46	0.0
<i>L. chalconica</i> 'White'	5	5	40	17.5	15	102	0.0
<i>L. chalconica</i> var. <i>alba</i>	4	4	47	8.5	25	100	1.0
Total for <i>L. chalconica</i>	5	7	178	25.8	31	1443	0.07
<i>L. xarkwrightii</i> 'Vesuvius'	6	11	69	65.2	27	1227	0.0
<i>L. xarkwrightii</i> 'Orange Genome'	3	3	7	28.6	39	77	0.0
Total for <i>L. xarkwrightii</i>	6	12	76	61.8	28	1304	0.0
<i>L. flos-cuculi</i>	4	4	30	6.7	2	4	0.0
<i>L. flos-cuculi</i> 'White Robin'	3	4	21	9.5	3	6	0.0
Total for <i>L. flos-cuculi</i>	5	6	51	7.8	3	10	0.0
<i>L. xhaageana</i> mixed hybrids	3	9	65	60.0	20	771	0.1
<i>L. xhaageana</i> 'Molten Lava'	3	6	16	81.3	34	443	0.9
<i>L. xhaageana</i> 'Lumina Salmon'	1	1	4	75.0	28	83	0.0
<i>L. xhaageana</i> 'Lumina Orange'	2	2	3	66.7	8	15	0.0
Total for <i>L. xhaageana</i>	4	10	88	67.0	23	1348	0.4
<i>L. cognata</i>	4	8	36	33.3	21	249	0.0
<i>L. miqueliana</i>	5	13	45	46.7	31	660	0.0
<i>L. wilfordii</i>	6	9	72	55.6	15	607	0.0

Table 5. Flower pollination, seed set, and germination for interspecific crosses excluding crosses in Table 6 as the pollen parent within *Lychnis*.

Pollen parent	No. of species mating	No. of cultivars mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalconica</i>	5	6	50	66.0	32	1054	0.0
<i>L. chalconica</i> 'Burning Love'	2	2	2	50.0	7	7	0.0
<i>L. chalconica</i> 'Carnea'	3	4	19	63.2	27	321	0.0
<i>L. chalconica</i> 'Rauhreif'	4	5	14	42.9	35	211	0.0
<i>L. chalconica</i> 'White'	5	6	20	70.0	23	320	0.0
<i>L. chalconica</i> var. <i>alba</i>	5	6	30	70.0	17	354	0.3
Total for <i>L. chalconica</i>	5	9	135	64.3	30	2348	0.04
<i>L. xarkwrightii</i>	1	1	2	100.0	44	87	0.0
<i>L. xarkwrightii</i> 'Vesuvius'	5	10	72	36.1	17	448	0.0
<i>L. xarkwrightii</i> 'Orange Genome'	1	1	1	0.0	0	0	0.0
Total for <i>L. xarkwrightii</i>	6	11	75	37.3	19	535	0.0
<i>L. flos-cuculi</i>	4	6	26	11.5	9	27	0.0
<i>L. flos-cuculi</i> 'White Robin'	2	3	10	30.0	7	21	0.0
Total for <i>L. flos-cuculi</i>	4	6	36	16.7	8	48	0.0
<i>L. xhaageana</i> mixed hybrids	3	8	79	26.2	33	569	0.2
<i>L. xhaageana</i> 'Molten Lava'	2	2	10	50.0	35	174	0.0
<i>L. xhaageana</i> 'Lumina Salmon'	2	2	2	0.0	0	0	0.0
Total for <i>L. xhaageana</i>	4	9	91	30.3	34	792	0.1
<i>L. cognata</i>	6	11	60	35.0	30	635	0.0
<i>L. miqueliana</i>	6	14	118	41.5	24	1177	0.3
<i>L. wilfordii</i>	6	7	31	12.9	10	38	0.0

data for each species, *L. flos-cuculi* and *L. wilfordii* produced relatively few seed setting flowers, seeds per capsule, and number of germinated seeds. The results indicated that some interspecific cross-compatibility barriers prevented hybridization and obtaining normal seeds from crossing combinations including *L. chalconica* × *L. cognata*, *L. chalconica* × *L. miqueliana*, *L. chalconica* × *L. xarkwrightii*, *L. chalconica* × *L. xhaageana*, *L. wilfordii* × *L. chalconica*, *L. cognata* × *L. flos-cuculi*, *L. cognata* × *L. miqueliana*, *L. cognata* × *L. wilfordii*, *L. miqueliana* × *L. wilfordii*, *L. miqueliana* × *L. xarkwrightii*, *L. flos-cuculi* × *L. miqueliana*, *L. miqueliana* × *L. xhaageana*, *L. flos-cuculi* × *L.*

xarkwrightii, *L. wilfordii* × *L. xarkwrightii*, *L. wilfordii* × *L. xhaageana*, *L. wilfordii* × *L. flos-cuculi*, and *L. flos-cuculi* × *L. xhaageana*. Nevertheless, because most of the crossing combinations produced immature seeds, embryo rescue techniques with immature seed culture might overcome the barriers and produce hybrid seedlings.

For the interspecific crosses among *Lychnis* species, there were significant differences for seed set between crossing combinations and their reciprocal crosses (Tables 4, 5, and 6). Therefore, crossing two interspecific parents in both directions would be a practical method to partially overcome cross-incompatibility between two species

in *Lychnis*. F₁ hybrid plants from 11 cross-compatible combinations among *L. cognata*, *L. xarkwrightii*, *L. xhaageana*, and from *L. xhaageana* 'Molten Lava' × *L. miqueliana* had intermediate ornamental traits like flower color, stem color, stem hardness, and leaf color between both parents (Tables 7 and 8). These phenotypes showed that promising hybrid plants could be obtained from interspecific crosses, although their hybrid identities need to be evaluated further by molecular marker methods or by observing segregation patterns in F₂ generation. For *L. xarkwrightii* 'Vesuvius' × *L. chalconica* var. *alba*, *L. chalconica* var. *alba* × *L. xhaageana* mixed hybrids,

Table 6. Flower pollination, seed set, and germination for interspecific crosses between *Lychnis cognata* and *L. xarkwrightii*, or *L. xhaageana*.

Seed parent	Pollen parent	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. cognata</i>	<i>L. xarkwrightii</i> 'Vesuvius'	7	57.1	27	109	77.1
<i>L. cognata</i>	<i>L. xhaageana</i> mixed hybrids	10	80.0	42	292	68.5
<i>L. cognata</i>	<i>L. xhaageana</i> 'Molten Lava'	5	100.0	19	77	53.2
<i>L. xarkwrightii</i> 'Vesuvius'	<i>L. cognata</i>	16	93.8	44	656	69.2
<i>L. xhaageana</i> mixed hybrids	<i>L. cognata</i>	20	60.0	42	458	75.3
<i>L. xhaageana</i> 'Molten Lava'	<i>L. cognata</i>	4	100.0	68	204	87.3
<i>L. xhaageana</i> 'Lumina Orange'	<i>L. cognata</i>	1	100.0	25	25	68.0
<i>L. xhaageana</i> 'Lumina Bronze Leaf Red'	<i>L. cognata</i>	1	100.0	11	11	64.0
<i>L. xarkwrightii</i> 'Vesuvius'	<i>L. xhaageana</i> mixed hybrids	5	40.0	42	84	60.7
<i>L. xhaageana</i> mixed hybrids	<i>L. xarkwrightii</i> 'Vesuvius'	8	87.5	39	274	68.2

Table 7. Flower color in parents and their hybrids for interspecific crosses in *Lychnis*.

Maternal color ^a	Maternal parent	Paternal color	Paternal parent	Hybrid color
30B	<i>L. xarkwrightii</i> 'Vesuvius'	White	<i>L. chalconica</i> var. <i>alba</i>	33B
White	<i>L. chalconica</i> var. <i>alba</i>	44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	White
44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	White	<i>L. chalconica</i> var. <i>alba</i>	43A
37A/37B	<i>L. cognata</i>	30B	<i>L. xarkwrightii</i> 'Vesuvius'	30A/30B/31C
30B	<i>L. xarkwrightii</i> 'Vesuvius'	37A/37B	<i>L. cognata</i>	30A/30B/31C/32A/32B/33B/40D/41C
37A/37B	<i>L. cognata</i>	44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	39A/40B/40D/43A/43B
44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	37A/37B	<i>L. cognata</i>	32B/33C/39A/40A/43C
37A/37B	<i>L. cognata</i>	44B	<i>L. xhaageana</i> 'Molten Lava'	43B/43C/40D
44B	<i>L. xhaageana</i> 'Molten Lava'	37A/37B	<i>L. cognata</i>	43A
30B	<i>L. xarkwrightii</i> 'Vesuvius'	44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	33B/47A
44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	30B	<i>L. xarkwrightii</i> 'Vesuvius'	32A/33A/33B/40A
30B/44B ^y	<i>L. xhaageana</i>	37A/37B	<i>L. cognata</i>	30B/32A/33B/40D/41C/43A
44B	<i>L. xhaageana</i> 'Molten Lava'	30A	<i>L. miqueliana</i>	40D
44B	<i>L. xhaageana</i> 'Molten Lava'	44B/30B ^y	<i>L. xhaageana</i> mixed hybrids	32A/43A
30B	<i>L. xarkwrightii</i> 'Vesuvius'	37A/37B	<i>L. cognata</i>	30A

^aFlower color recorded according to The Royal Horticultural Society Color Chart (1966).^yFlower colors in this species are with many variations.Table 8. Morphological assessments for six selected interspecific hybrids in *Lychnis*.

Hybridization ^a	Corolla diam (cm) ^y	Petal length (cm) ^y	Petal width (cm) ^y	Flower color ^x	Leaf color ^x
<i>L. xarkwrightii</i> 'Vesuvius'	5.80 a	2.76 a	2.14 a	33A	147A
<i>L. chalconica</i> var. <i>alba</i>	1.80 c	0.84 c	0.70 c	White	146A
Hybrids	3.66 b	1.78 b	1.82 b	33B	147B
LSD _{0.05}	0.32	0.19	0.25		
<i>L. chalconica</i> var. <i>alba</i>	1.80 b	0.84 b	0.70 b	White	146A
<i>L. xhaageana</i> mixed hybrids	4.22 a	1.96 a	1.78 a	44B	147A
hybrids	1.28 c	0.58 c	0.48 c	White/56D	146C
LSD _{0.05}	0.40	0.18	0.14		
<i>L. xhaageana</i> mixed hybrids	4.22 a	1.96 a	1.78 a	44B	147A
<i>L. chalconica</i> var. <i>alba</i>	1.80 c	0.84 c	0.70 c	White	146A
Hybrids	3.00 b	1.58 b	1.48 b	43A	144A
LSD _{0.05}	0.35	0.20	0.17		
<i>L. xarkwrightii</i> 'Vesuvius'	5.80 a	2.76 a	2.14 a	30B	147A
<i>L. cognata</i>	5.22 b	2.34 b	1.94 a	37A/37B	146A/147B
Hybrids	5.56 ab	2.58 ab	2.06 a	40D/30A/30B	137B
LSD _{0.05}	0.38	0.22	NS		
<i>L. cognata</i>	5.22 b	2.34 b	1.94 b	37A/37B	146A/147B
<i>L. xhaageana</i> 'Molten Lava'	4.60 c	2.24 b	2.02 b	44B	147A/147B
hybrids	6.00 a	2.76 a	2.74 a	43B/43C/40D	146A/147A
LSD _{0.05}	0.40	0.18	0.31		
<i>L. xhaageana</i> 'Molten Lava'	4.60 b	2.24 b	2.02 b	44B	147A/147B
<i>L. cognata</i>	5.22 a	2.34 b	1.94 b	37A/37B	146A/147B
Hybrids	5.66 a	2.64 a	2.38 a	33A/33B/34A; 43A/43B/43C	146A/147B
LSD _{0.05}	0.58	0.27	0.34		

^aMaternal plant followed by paternal plant.^yDifferent letters in one column indicate means significantly different at $P \leq 0.05$, $n = 5$.^xFlower and leaf color recorded according to The Royal Horticultural Society Color Chart (1966).

LSD = least significant difference; NS = nonsignificant.

and *L. xhaageana* mixed hybrids $\times$ *L. chalconica* var. *alba*, progeny flower color was more similar to the maternal parent.

Intergeneric crosses. Because of cross-incompatibility, crossing combinations between *Lychnis* spp. and *Silene* spp. had the lowest number of seed setting flowers and seeds. Of 290 crosses made, only 39 set seed

(Tables 9 and 10). Partial compatible crossing combinations were made from *L. chalconica* $\times$ *S. armeria*, *L. cognata* $\times$ *S. plankii* and its reciprocal cross, *L. cognata* $\times$ *S. pendula*, *S. plankii* $\times$ *L. miqueliana*, *L. xarkwrightii* 'Vesuvius' $\times$ *S. armeria*, *L. xarkwrightii* 'Vesuvius' $\times$ *S. pendula*, *S. plankii* $\times$ *L. xarkwrightii* 'Vesuvius', *L.*

xhaageana mixed hybrids $\times$ *S. armeria*, *L. xhaageana* mixed hybrids $\times$ *S. pendula*, *L. xhaageana* 'Molten Lava' $\times$ *S. pendula*, *L. wilfordii* $\times$ *S. armeria*, and *L. wilfordii* $\times$ *S. pendula*. Among intergeneric crossing combinations, seed set rates varied. No seeds germinated; thus, embryo rescue techniques with immature seed culture would likely

Table 9. Flower pollination, seed set, and germination for intergeneric crosses as seed parent between *Lychnis* and *Silene*.

Seed parent	No. of species mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalcedonica</i>	1	6	50.0	12	37	0.0
<i>L. chalcedonica</i> 'Burning Love'	1	6	0.0	0	0	0.0
<i>L. chalcedonica</i> 'White'	2	10	0.0	0	0	0.0
<i>L. chalcedonica</i> var. <i>alba</i>	2	11	0.0	0	0	0.0
Subtotal for <i>L. chalcedonica</i>	2	33	9.1	12	37	0.0
<i>L. xarkwrightii</i> 'Orange Genome'	1	3	0.0	0	0	0.0
<i>L. xarkwrightii</i> 'Vesuvius'	3	18	22.2	9	35	0.0
Subtotal for <i>L. xarkwrightii</i>	3	21	19.0	9	35	0.0
<i>L. flos-cuculi</i> 'White Robin'	1	5	0.0	0	0	0.0
<i>L. xhaageana</i> mixed hybrids	4	34	176	86	45	0.0
<i>L. xhaageana</i> 'Molten Lava'	1	3	100.0	14	41	0.0
<i>L. cognata</i>	3	25	28.0	12	82	0.0
<i>L. miqueliana</i>	3	11	0.0	0	0	0.0
<i>L. wilfordii</i>	3	24	20.8	7	33	0.0
Total for genus <i>Lychnis</i>	4	156	17.9	10	273	0.0
<i>S. armeria</i>	7	77	0.0	0	0	0.0
<i>S. delavayi</i>	1	1	0.0	0	0	0.0
<i>S. plankii</i>	4	21	52.4	36	394	0.0
<i>S. pendula</i>	6	35	0.0	0	0	0.0
Total for genus <i>Silene</i>	7	134	8.2	36	394	0.0

Table 10. Flower pollination, seed set, and germination for intergeneric crosses as pollen parent between *Lychnis* and *Silene*.

Pollen parent	No. of species mating	No. of cross-pollinated flowers	Percent of flowers setting seed	Avg seed no. per capsule	Total no. of seeds	Percent germination
<i>L. chalcedonica</i>	2	12	0.0	0	0.0	0.0
<i>L. chalcedonica</i> 'Burning Love'	1	1	0.0	0	0.0	0.0
<i>L. chalcedonica</i> 'Carnea'	1	6	0.0	0	0.0	0.0
<i>L. chalcedonica</i> var. <i>alba</i>	2	12	0.0	0	0.0	0.0
Subtotal for <i>L. chalcedonica</i>	2	31	0.0	0	0.0	0.0
<i>L. xarkwrightii</i>	1	1	0.0	0	0.0	0.0
<i>L. xarkwrightii</i> 'Vesuvius'	3	23	8.7	30	60	0.0
Subtotal for <i>L. xarkwrightii</i>	4	24	8.3	30	60	0.0
<i>L. flos-cuculi</i>	1	2	0.0	0	0	0.0
<i>L. xhaageana</i> mixed hybrids	2	32	0.0	0	0	0.0
<i>L. cognata</i>	3	19	31.6	39	232	0.0
<i>L. miqueliana</i>	3	15	20.0	34	102	0.0
<i>L. wilfordii</i>	3	11	0.0	0	0	0.0
Total for genus <i>Lychnis</i>	4	134	8.2	36	394	0.0
<i>S. acaulis</i> ssp. <i>acaulescens</i>	1	2	0.0	0	0	0.0
<i>S. armeria</i>	6	75	9.3	6	45	0.0
<i>S. plankii</i>	6	29	6.9	4	8	0.0
<i>S. pendula</i>	6	50	38.0	12	220	0.0
Total for genus <i>Silene</i>	7	156	17.9	10	273	0.0

have to be used to produce seedlings. Again, seed set differences in reciprocal crosses helped to partially overcome interspecific cross-incompatibility between two genera.

Selfing rate. According to our observation, *L. chalcedonica* and *L. flos-cuculi* have a very high selfing rate. *Lychnis wilfordii* yielded a very low selfing rate in the greenhouse despite producing a lot of pollen. No hybrid plants were obtained from *L. wilfordii*, yet the profuse flowering makes it a potential parent for ornamental breeding through embryo rescue.

Breeding potentials. The common name of the family Caryophyllaceae is carnation

family or pink family; however, pink color is not a prevailing color in *Lychnis* species used in the hybridizations. Two species, *L. miqueliana* and *L. cognata*, are pink with relatively large flowers making them good potential parents, but both had weak stems when cultivated in the greenhouse. *Lychnis miqueliana* did not readily produce hybrids with other species. The interspecific hybridizations between *L. cognata* and other *Lychnis* species were successful. Six hybrids generated from *L. cognata* were pink (Table 7). The stem color of the hybrids between *L. xarkwrightii* 'Vesuvius' and *L. cognata* was partially inherited from 'Vesuvius'

showing reddish internodes. The stems were stronger than *L. cognata*. *Lychnis cognata* proved to be a good parent for crossing for its promising interspecific cross-compatibility and pink flower color. In this study, at least 11 interspecific hybrids with rich variations in flower color, stem type, stem color, and leaf color were generated without the need for embryo rescue. Six promising hybrids were selected according to their attractive ornamental phenotypes and morphology (Table 8). To expand genetic variations for ornamental flower breeding programs using *Lychnis* and *Silene*, immature seed culture protocols, phenotype selection, fertility

status, and propagation protocols for hybrids need to be investigated further.

Discussion

Hybridization at intraspecific, interspecific, and intergeneric levels is commonly practiced in breeding programs to combine desirable traits. Our results demonstrated that mature seeds and normal plants can be produced from intraspecific hybridization within *L. chalconica* and *L. xhaageana* species, confirming results by Nakano et al. (2013). In their study, Nakano et al. (2013) made cross-pollinations among eight species with the same chromosome number ($2n = 2x = 24$) in *Lychnis*, including *L. chalconica*, *L. coronata*, *L. fulgens*, *L. gracillima*, *L. kiusiana*, *L. miqueliana*, *L. sieboldii*, and *L. wilfordii*; based on the average value of each species, *L. coronata* yielded both the lowest seed set (8.3%) and seed number with four per capsule. Seed set for the other seven species ranged from 50.6% to 81.5%, and seed number per capsule ranged from seven to 55. However, only immature seeds were obtained from these interspecific crossing combinations 4 weeks after pollination. By immature seed culture on half-strength Murashige and Skoog medium without plant growth regulators, of a total of 26 crosses that produced seeds, seeds from 11 crosses germinated in 3 months, and only one of them produced seedlings. Compared with the previously mentioned report, we used different species and many cultivars in some species to test crossing compatibility for interspecific crosses in *Lychnis*. Also, the number of crosses including reciprocal crosses in our study was higher. Based on our study, high seed set and germination percentages were observed from the interspecific crossing combination among *L. cognate*, *L. xarkwrightii*, and *L. xhaageana* with no cross-incompatible barriers existing between them. For most of the other interspecific crosses, our results were similar to Nakano et al.'s report (Nakano et al., 2013). For those crosses that only have immature seeds, embryo rescue methods

need to be developed to produce hybrid plants. Also, the fertility and propagation procedure of interspecific hybrids should be investigated further.

Although all plants have the same chromosome number, cross-compatibility and hybrid seed germination between *Lychnis* and *Silene* were significantly lower than those between species in the genus *Lychnis*. The production of F_1 hybrids showed some indication that *Lychnis* and *Silene* are closely related. This breeding evidence seems to support the conclusion that *Lychnis* and *Silene* are two genera instead of one genus classification (Rabeler, 1992). However, considering the large number of species in *Silene* and their broad distribution, the generic delimitation of *Silene* still needs to be studied in detail.

Because a significant number of crosses in a blooming season was performed with limited personnel in this study, hand hybridization without emasculation was conducted to save time and labor. Crosses are sometimes made without emasculation such as Yang et al. (2010) who conducted intergeneric hybridization without emasculation between *Opisthopappus taihangensis* (Ling) C. Shih. and *Chrysanthemum lavandulifolium* (Fisch. ex Trautv.). From our observation in the greenhouse, *Lychnis* is protandry, which facilitates non-emasculation if large hybrid populations are wanted with limited personnel. Genotype fingerprinting tools could be used to verify hybridity (Nakano et al., 2013). Pollen storage and embryo rescue techniques would facilitate a greater number of intraspecific, interspecific, and intergeneric crosses and hybrids.

Literature Cited

- Desfeux, C. and B. Lejeune. 1996. Systematics of euromediterranean *Silene* (Caryophyllaceae): Evidence from a phylogenetic analysis using ITS sequences. *Cr. Acad. Sci. Iii-Vie* 319:351–358.
- Godo, T., J. Miyazaki, S. Kuwayama, S. Ogita, Y. Kato, M. Nakano, and M. Nakata. 2009. Interspecific hybridization between triploid Senno (*Lychnis senno* Siebold et Zucc., Caryophyllaceae) and allied taxa of the genus *Lychnis*. *Plant Biotechnol.* 26:301–305.
- Kruckeberg, A. 1962. Intergeneric hybrids in the lychnideae (Caryophyllaceae). *Brittonia* 14: 311–321.
- Mayol, M. and J.A. Rossello. 2006. Hybridization studies in *Silene* subgen. petrocopsis (Caryophyllaceae). *Folia Geobot.* 41:203–212.
- Melzheimer, V. 1988. *Silene*. In: Rechinger, K.H. (ed.). *Flora iranica* Lfg. Akademische Druck- & Verlagsanstalt, Graz. 163:341–508.
- Nakano, M., S. Kuwayama, E. Oka, M. Asano, D. Han, and T. Godo. 2013. Cross-compatibility in interspecific hybridization of *Lychnis* (Caryophyllaceae) and characterization of interspecific hybrids between *L. fulgens* and *L. sieboldii*. *J. Jpn. Soc. Hort. Sci.* 82:57–62.
- Negrutiu, I., B. Vyskot, N. Barbacar, S. Georgiev, and F. Moneger. 2001. Dioecious plants. A key to the early events of sex chromosome evolution. *Plant Physiol.* 127:1418–1424.
- Oxelmann, B., M. Lidén, R.K. Rabeler, and M. Popp. 2000. A revised classification of the tribe Sileneae (Caryophyllaceae). *Nord. J. Bot.* 20:743–748.
- Popp, M., A. Gizaw, S. Nemomissa, J. Suda, and C. Brochmann. 2008. Colonization and diversification in the African 'Sky Islands' by Eurasian *Lychnis* L. (Caryophyllaceae). *J. Biogeogr.* 35:1016–1029.
- Rabeler, R.K. 1992. Proposal to conserve 2490 *Silene* L. against *Lychnis* L. (Caryophyllaceae). *Taxon* 41:126–128.
- Royal Horticultural Society. 1966. Royal Horticultural Society colour chart. Royal Hort. Soc., London, and Flower Council of Holland, Leiden.
- Walters, S.M. 1989. *Silene*; *Lychnis*, p. 179–182. In: Walters, S.M., J.C.M. Alexander, A. Brady, C.D. Brickell, J. Cullen, P.S. Green, V.H. Heywood, V.A. Matthews, N.K.B. Robson, P.E. Yeo, and S.G. Knees (eds.). *The European garden flora*, 3. Cambridge University Press, Cambridge, UK.
- Wilson, G.B., J. Wright, P. Lusby, W.J. Whittington, and R.N. Humphries. 1995. *Lychnis viscaria* L. (*Viscaria vulgaris* Bernh.). *J. Ecol.* 83:1039–1051.
- Yang, D.Y., X. Hu, Z.H. Liu, and H.E. Zhao. 2010. Intergeneric hybridizations between *Opisthopappus taihangensis* and *Chrysanthemum lavandulifolium*. *Sci. Hort.* 125:718–723.