Interspecific Hybridization of *Fragaria vesca* subspecies with *F. nilgerrensis*, *F. nubicola*, *F. pentaphylla*, and *F. viridis*

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ABSTRACT. The potential of using *Fragaria vesca* L. as a bridge species for interspecific hybridization to *F. nilgerrensis* Schlecht, *F. nubicola* Lindl., *F. pentaphylla* Losinsk, and *F. viridis* Duch. was investigated using a wide germplasm base of 40 *F. vesca* accessions. This study was successful in producing many hybrids between *F. vesca* and other diploid species indicating its value as a bridge species. Of the species used as males, *F. nubicola*, *F. pentaphylla*, and *F. viridis* accessions were more successful, averaging 8 to 16 fruit and 16 to 25 seeds/fruit. It was most difficult to obtain hybrids with *F. nilgerrensis*, which had only three seeds per fruit. Differences among pollen donors were minimal when hybrid seeds were germinated in vitro. For different species combinations, 75% to 99% of seeds had embryos and 77% to 89% of these embryos germinated. The lack of significant differences in crossability variables among the four *F. vesca* subspecies [i.e., ssp. *americana* (Porter) Staudt, ssp. *bracteata* (Heller) Staudt, ssp. *vesca* L., and ssp. *vesca* var. *semperflorens* L.] demonstrated the similarity between these species and the strong potential for gene flow between *F. vesca* and other diploid species. As European and North American *F. vesca* subspecies are not sufficiently divergent to differ in interspecific hybridization, *F. vesca* may be a younger species rather than an older progenitor species.

The synthetic octoploid (SO) system (Evans, 1977) was developed as a means to incorporate germplasm of 2x, 4x, and 6x *Fragaria* species into octoploid hybrids. This system bypasses ploidy level differences and facilitates introgression of 2x, 4x, and 6x species into the cultivated strawberry (*Fragaria ×ananassa* Duch.) via the synthetic octoploid (Evans, 1977). The first step of a synthetic octoploid system is the intercrossing of diploid species to create diploid hybrids (Evans, 1977).

The use of a bridge species (or pivotal genome) to facilitate interspecific crosses has been valuable for introgressing diploid species into polyploid cereals (Gustafson and Dera, 1989; Gustafson and Sears, 1993) but no suggestions for bridge species have been mentioned in previous investigations of SO systems with *Fragaria* (Evans, 1977; Sebastampillia and Jones, 1976). *Fragaria vesca* is a logical choice as a bridge species for crosses with other diploid *Fragaria* species in the initial stage of the SO system because it has been successfully hybridized with several *Fragaria* species including octoploid cultivars (Ahmadi and Bringhurst, 1992; Evans, 1964, 1974, 1977; Evans and Jones, 1967; Fadeeva, 1966; Federova, 1946; Sebastampillia and Jones, 1976; Senanayake and Bringhurst, 1967; Staudt, 1959, 1967, 1989; Trajkovski, 1993; Williams, 1959; Yarnell, 1931), may be progenitor of other *Fragaria* species (Bringhurst and Gill, 1970; Duchesne in Darrow, 1966; Longley in Darrow, 1966; Nurnberg-Kruger, 1958; Staudt, 1953) and may, therefore, be closely related to other diploid species. *Fragaria vesca* may be best utilized as a female parent to overcome incompatibility mechanisms in some diploid species (Evans and Jones, 1967). The chloroplast genome of *F. vesca* has greater genetic diversity compared to other *Fragaria* species (Harrison et al., 1993, 1997), which may be important to the success of interspecific hybridization (Stoskopf et al., 1993).

In addition, *F. vesca* cytoplasm and genomes are more amenable than other *Fragaria* species to chromosome doubling by colchicine (Fadeeva, 1966; Sebastampillai and Jones, 1976), another important consideration in the SO system.

If *F. vesca* is the progenitor to other diploid species (Duchesne in Darrow, 1966; Longley in Darrow, 1966; Nurnberg-Kruger; 1958; Staudt, 1953), then *F. vesca* subspecies may differ in relatedness and, hence, crossability with other diploid species. With a range of distribution over five continents, *F. vesca* subspecies could have become geographically isolated and diverged sufficiently to differ in interspecific crossability. At least one *F. vesca* population in Neb. was unique due to its isolation (Potter et al., 2000). Although it has been suggested that *F. vesca* is not highly variable genetically (Potter et al., 2000; Reed, 1966), there have been several studies that have highlighted morphological differences of plant form, fruit, leaves and flowers of *F. vesca* subspecies (Ahmadi and Bringhurst, 1990; Harrison et al., 1993, 1997). *Fragaria vesca* subspecies also differed in chloroplast DNA restriction fragment length polymorphism markers (Harrison et al., 1997), the number of genes governing photosensitivity (Ahmadi and Bringhurst, 1990) and sex expression (Ahmadi and Bringhurst, 1991). Several subspecies need to be included in any studies to determine if these differences impact on interspecific hybridization.

Fragaria vesca's adaptation to low light levels (Chabot and Chabot, 1977) and cool temperatures (Bauer, 1976) may be especially useful for breeding cultivars for northern temperate areas and enhanced late season growth (Bauer, 1976), flower bud initiation in the fall, and yield potential if these adaptations were successfully transferred into cultivars. *Fragaria vesca* fruit also has a very desirable aroma that could be used to improve fruit quality of modern cultivars (Ahmadi and Bringhurst, 1992). The use of *F. vesca* subspecies in interspecific crosses in this study may also aid in understanding divergence and evolution in *Fragaria*. Since *F. vesca* is distributed across five continents with broad adaptation to diverse conditions (Darrow, 1966) crossability differences among *F. vesca* subspecies could also be exploited to optimize an SO system.

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In this study, *F. vesca* ssp. *americana*, ssp. *bracteata*, ssp. *vesca*, and ssp. *vesca* var. *semperflorens* are examined for differences in crossability. The latter group, ssp. *vesca* var. *semperflorens*, is the cultivated form of *F. vesca*, which has diverged through artificial selection.

The objective of this study is to determine the crossability of four *F. vesca* subspecies with the accessions of four other diploid species. In addition, several accessions within a subspecies were used to investigate the potential divergence of species. This information could be important for optimizing a crossing strategy for the early stages of the SO system (Evans, 1977) and may provide insight into relationships between *Fragaria* species.

Material and Methods

GERMPLASM AND CROSSING DESIGN. The *F. vesca* female parents used in this study (Table 1) were grouped by subspecies (ssp. *americana*, ssp. *bracteata*, ssp. *vesca*, or ssp. *vesca* var. *semperflorens*). One *F. vesca* accession was used as a pollen source for control crosses and six male parents [i.e., *F. nilgerrensis* (nil1), *F. nubicola* (nub1, nub2), *F. pentaphylla* (pen1), and *F. viridis* (vir1 and vir2) (Table 2)] were used for interspecific crosses. Ten *F. vesca* var. *semperflorens* cultivars were assigned to each of the three blocks but accessions of the three wild subspecies (i.e., ssp. *americana*, ssp. *bracteata*, ssp. *vesca*) were randomly distributed among the blocks.

Controlled crosses with *F. vesca* pollen were done to verify that emasculation and pollen storage techniques were correct and for comparison with interspecific crosses. VW69 was used for control crosses because it was native to Asia (unlike other *F. vesca* accessions in the study) and produced abundant pollen. The number of male/female combinations was maximized by crossing the genotypes within blocks. Each female genotype (Table 1) was pollinated with up to seven accessions for interspecific crosses. Data from control crosses are discussed in results but are not included in the data set of interspecific crosses. The recessive everbearing and nonrunner traits of var. *semperflorens* cultivars were useful as morphological markers to easily identify true hybrids from selfs.

Crosses were done over a period of several months as different genotypes flowered. An attempt was made to pollinate each female with an equal number of the different species that were used as pollen donors. Choice of parents was made to maximize the number of accession combinations and, thus, sample the greatest amount of diversity.

HANDLING OF PARENT PLANTS. In early November, dormant plants were dug from the field and stored bare root in a freezer at -1°C . From late December to early March, plants were potted with a soilless mix (Promix BX; Premier Horticulture, Red Hill, Pa.) in either 3- or 6-L pots (Plant Products, Brampton, Ont.) and grown in the greenhouse at Guelph, Ont. Plants were watered as required with deionized water and fertilized with fertilizer solution [20N–3.5P–16.6K (Plant Products, Brampton, Ont.) at a concentration of $267\text{ mg}\cdot\text{L}^{-1}$ with a pH of 6.0 phosphoric acid]

FLORAL INDUCTION. Some *F. nilgerrensis*, *F. nubicola*, *F. pentaphylla* and *F. viridis* did not bloom under the greenhouse conditions described above. They required additional short day and temperature treatments [4 weeks, 10-h photoperiod, 18°C day/ 15°C night temperature in a growth chamber (model EY8M; Controlled Environments, Winnipeg, Man.)] to induce flowering. The flower induction treatment was applied in late winter or early spring to greenhouse plants that showed no signs of blooming.

Table 1. *Fragaria vesca* accessions used as female parents for interspecific hybridization with four diploid *Fragaria* species. Accessions are grouped by subspecies.

Genotype and Guelph identification code	Species and accession number from original source	Source of germplasm ²
<i>F. vesca</i> ssp. <i>vesca</i> var. <i>semperflorens</i> (day-neutral cultivars)		
vcv02	PI 551517, Alpine	NCGR
vcv04	PI 551827, Yellow Wonder	NCGR
vcv06	CFRA1185.001, Golden Alpine	NCGR
vcv07	Pineapple Crush	Park Seed
vcv10	Alexandria	Thompson & Morgan
vcv11	Ruegen	Richters Seeds
vcv13	Mignonette	Park Seed Co.
vcv14	Golden Sprite	Ornamental Edibles
vcv20	Alpine Strawberry	White Flower Farms
vcv22	FRA 2193	IPK Genbank
<i>F. vesca</i> ssp. <i>vesca</i> (wild type from Europe)		
vw 07	PI 551792	NCGR
vw 09	PI 551890	NCGR
vw10	PI 551892	NCGR
vw11	PI 551893	NCGR
vw12	PI 551909	NCGR
vw13	PI 552273	NCGR
vw14	PI 552274	NCGR
vw16	PI 552239	NCGR
vw22	FRA 13197	IPK Genbank
vw38	PI 552242	NCGR
<i>F. vesca</i> ssp. <i>bracteata</i> (wild type from western North America)		
vw 02	PI 551519	NCGR
vw 06	PI 511791	NCGR
vw23	PI 551524	NCGR
vw24	PI 551551	NCGR
vw25	PI 551644	NCGR
vw26	PI 551645	NCGR
vw57	PI 148	CCG
vw58	PI 418	CCG
<i>F. vesca</i> ssp. <i>americana</i> (wild type from eastern North America)		
vw17	PI 552246	NCGR
vw29	PI 552241	NCGR
vw30	PI 552247	NCGR
vw32	PI 552286	NCGR
vw33	Grand River, Cambridge, Ont.	Spencer Murch
vw34	Cambridge, Ont.	collected by author
vw35	PI 552243	NCGR
vw36	PI 552244	NCGR
vw47	PI 551898	NCGR
vw48	PI 551908	NCGR
vw49	Smithfield, Ont.	collected by author
vw53	Newcastle, Ont.	collected by author

²National Clonal Germplasm Repository (NCGR), Corvallis, Ore.; Park Seed Co. Greenwood, S.C.; Thompson & Morgan, Jackson, N.J.; Richters Herbs, Goodwood, Ont.; Ornamental Edibles, San Jose, Calif.; White Flower Farm, Litchfield, Conn.; Inst. für Pflanzengenetik und Kulturpflanzenforschung (IPK Genbank), Gatersleben, Germany; Canadian Clonal Genebank (CCG), Agriculture and Agri-Food Canada, Harrow, Ont.

Table 2. *Fragaria* species accessions used as pollen donors in crosses with *F. vesca*.

Species	Accession no. from source	Accession code for this study	Source ^z
<i>F. nilgerrensis</i>	CFRA 1188.000	nil 1	NCGR
<i>F. nubicola</i>	PI 551851	nub1	NCGR
	PI 551853	nub 2	NCGR
<i>F. pentaphylla</i>	PI 881143	pen 1	RBG
<i>F. viridis</i>	PI 551741	vir 1	NCGR
	PI 551742	vir 2	NCGR

^zNational Clonal Germplasm Repository (NCGR), Corvallis, Ore.; Royal Botanical Garden (RBG), Edinburgh, Scotland.

POLLINATION PROCEDURES. All flowers were emasculated prior to crossing. A method to store pollen to facilitate crossing was adapted from MacFarlane Smith et al. (1989). Pollen was stored with a desiccant at 3 to 4 °C.

SEED COLLECTION AND STORAGE. Ripe fruit were either crushed on petri dishes or placed intact into airtight jars containing desiccant. Crushed fruit were air-dried at room temperature. Once dry, achenes were rubbed off, counted and stored at room temperature or in a refrigerator at 3 to 5 °C. Only large plump seed were collected and stored. Intermediate and small-sized seeds were assumed to contain no embryos based on previous investigations (unpublished data).

IN VITRO GERMINATION. The in vitro germination technique of Miller et al. (1992) was modified for use in this research. This technique was originally developed for use with *F. xananassa* seed, but was adapted for seeds resulting from interspecific hybridizations. Murshige and Skoog medium (Sigma-Aldrich Canada, Oakville, Ont.) was prepared with no hormones as described by Miller et al. (1992) but Gellan gum agar (Schweizerhall, South Plainfield, N.J.) at 2.5 g·L⁻¹ was substituted for agar. Acid washed, activated charcoal (Gibco Laboratories/Life Technologies, Grand Island, N.Y.) was added at 3.0 g·L⁻¹ to darken the medium. In a previous study (data not reported), shoots of seedlings germinated on clear medium often grew inverted or laterally. Medium (7.5 mL) was placed in 30-mL glass French squares, autoclaved (model 3021; AMSCO, Erie, Pa.) at 121 °C for 20 min and allowed to solidify in a slanted position on racks.

For surface sterilization, achenes were wrapped in cheese cloth, placed into a 0.5% (v/v) sodium hypochlorite (Lilo Products, Hamilton, Ont.) solution containing Tween 20 (2.0 µL·L⁻¹) and placed on an orbital shaker (Labline Instruments, Melrose Park, Ill.) for 20–30 min. Agitation continued through three transfers into vials of sterile deionized water to remove the sodium hypochlorite solution. Achenes were placed on moistened sterilized filter paper to prevent desiccation, and the distal third of each achene was removed with a scalpel and discarded. Cutting occurred under a dissecting microscope (Aus JENA, Jena, Germany) in a laminar flow hood (Canadian Cabinets Co., Ottawa, Ont.). Cut achenes were replaced in vials on the surface of the medium described above. These vials were placed on racks and in a tissue culture growth room maintained at 20 °C, 30 cm below 40-W cool-white fluorescent lights (Sylvania, Mississauga, Ont.) at 70 µmol·m⁻²·s⁻¹ (photosynthetically active radiation). Seeds usually germinated and had fully expanded cotyledons within 10 d of being cut and placed on the media.

DATA ANALYSIS. The following traits were measured, fruit number, average number of seeds per fruit, seeds with embryos (percent) and germination (percent). Data were analyzed with the GLM program of SAS (SAS for Windows version 6.12; SAS

Inst., Cary, N.C.). Comparison of means was accomplished using the LS means function. Analysis of fruit number and seeds per fruit was based on the total number of accessions in a species combination, while seeds with embryos (percent) and germination (percent) were based on a subset of those combinations that received in vitro germination.

Results

The control crosses with *F. vesca* (vw69) verified that both emasculation and pollen storage techniques were successful (data not reported). The number of potential combinations of parents was limited by flowering times of different accessions within a species. There were 191 unique parental combinations achieved by 360 interspecific hybridizations. The four *F. vesca* subspecies were successfully hybridized with the four species used as the pollen parent. The species, nil1, nub1, nub2, pen1, vir1, and vir2 were used in 37, 38, 28, 27, 35, and 36 hybrid combinations, respectively. Pen1 produced trusses with only one to three flowers and was used in fewer combinations. Plants of ssp. *bracteata* had fewer flowers per plant than other *F. vesca* subspecies in the study so fewer crosses occurred with this group. Since data on the individual accessions were nonsignificant only means of the subspecies are presented. However among the many accessions used in this study, some had higher crossability indicators.

FRUIT NUMBER AND SEEDS PER FRUIT. Pollen donor species were a significant source of variation for fruit number and average seed number but *F. vesca* subspecies and the interaction sources of variation were not significant. The *F. nilgerrensis* accession, nil1, had the lowest fruit number, 39%, while other pollen donors averaged between 80% and 100% (Table 3). Crosses having the nil1 accession as a male also had the lowest total seed number (33). Species used as pollen donors (i.e., nub2, pen1, vir1, and vir2) produced seeds per fruit averaging from 15 to 20, while nub1 was highest with 25 seeds per fruit (Table 3).

The European subspecies *vesca* var. *semperflorens* produced the highest average fruit number (18) and was significantly higher than ssp. *bracteata*, a North American subspecies (Table 3).

SEEDS WITH EMBRYOS AND EMBRYO GERMINATION. Insufficient numbers of hybrid seeds were produced with *F. vesca* ssp. *bracteata* females to allow meaningful analysis of variance (ANOVA) of differences among *F. vesca* subspecies for the proportion of seeds with embryos and percent embryo germination. Therefore, only the effect of male accessions was incorporated into ANOVAs of these two variables (data not reported). Male accessions were significantly different for percent of seeds with embryos but not for in vitro embryo germination (Table 4). The percentage of seeds with embryos was greater than 75% for all accessions. Four species used as pollen donors were at least 13% higher than nil1 or vir2 for percentage of seeds with embryos (Table 4). Percent embryo germination (in vitro) was similar for all males, with only a 12% difference between the lowest (vir2) and highest (vir1) male.

After germination, all seedlings were allowed to reach maturity to confirm longer term viability and fertility. These hybrids were subsequently used in advanced phases of the synthetic octoploid system.

Discussion

This study used a far greater number (39) of *F. vesca* accessions than those used in other studies of interspecific hybridization of

Table 3. Number of crosses (N), fruit number and total number of seeds for crosses among *Fragaria vesca* subspecies when pollinated with *F. nilgerrensis* (nil 1), *F. nubicola* (nub 1 and 2), *F. pentaphylla* (pen 1), and *F. viridis* (vir1 and 2).

Species accessions used as pollen donors																					
F. vesca subspecies used as females	F. nilgerrensis (nil1)			F. nubicola (nub1)			F. nubicola (nub2)			F. pentaphylla (pen1)			F. pentaphylla (vir1)			F. viridis (vir2)			Mean ^z		
	Seeds			Seeds			Seeds			Seeds			Seeds			Seeds			Total	Fruit	Seeds
	Fruit	(no./		Fruit	(no./		Fruit	(no./		Fruit	(no./		Fruit	(no./		Fruit	(no./		N	(no.)	(no.)
	N	(no.)	fruit	N	(no.)	fruit	N	(no.)	fruit	N	(no.)	fruit	N	(no.)	fruit	N	(no.)	fruit	N	(no.)	(no.)
americana	26	7	4	8	8	27	10	8	14	7	7	17	31	22	19	14	12	20	96	11 ab	17 a
vesca	26	11	1	11	11	24	11	11	19	12	11	15	11	10	11	12	10	14	82	11 ab	14 a
vesca var. sempervlorens	45	23	4	10	10	22	12	9	18	8	8	17	27	26	13	34	33	6	136	18 a	13 a
bracteata	14	3	4	5	5	26	7	3	28	6	5	24	6	5	13	8	7	18	46	5 b	19 a
Mean ^y	28	11 a	3 c	9	9 a	25 a	10	8 a	20 ab	8	8 a	18 b	19	16 a	14 b	17	16 a	15 b			

^zMeans within a column followed by the same letter are not significantly different at $P = 0.05$.

^yMeans of the same trait within a row followed by the same letter are not significantly different at $P = 0.05$.

Table 4. Seeds with embryos (percent) and embryo germination (percent) for interspecific crosses among *Fragaria vesca* accessions used as female parents with four diploid species as pollen donors.

<i>Fragaria</i> species used as pollen donors	Accession code	Female <i>F. vesca</i> accessions used in crosses (no.)	Total seeds germinated in vitro (no.)	Seeds with viable embryos (%)	In vitro embryo germination (%)
<i>F. nilgerrensis</i>	nil1	15	108	75b ^z	85a
<i>F. nubicola</i>	nub1	12	324	97a	81a
	nub2	17	397	99a	84a
<i>F. pentaphylla</i>	pen1	21	490	92a	81a
<i>F. viridis</i>	vir1	11	241	99a	89a
	vir2	6	308	79ab	77a
Mean		14	311	90	82

^zMeans followed by the same letter are not significantly different at $P = 0.05$ level.

diploid *Fragaria*. The current study thus provides a better representation of the diversity within *F. vesca* compared to earlier studies. *F. vesca* subspecies did not differ statistically in interspecific crossability in this study although considerable variation for crossability was present among the accessions within a subspecies (data not reported). The interactions among *F. vesca* subspecies and four other species were not statistically different for any of the variables investigated. The hypothesis that *F. vesca* subspecies differ in interspecific crossability (Fadeeva, 1966; Williams, 1959) was not supported statistically by the results when averaged over a large number of accessions. Since the genotypes within each of the *F. vesca* subspecies ranged widely in crossability, it is quite possible that smaller collections of *F. vesca* subspecies commonly used in previous studies could skew results leading to the erroneous conclusion that some subspecies were superior. For example, in a past study (Fadeeva, 1966) the conclusion that cultivars of *F. vesca* var. *semperflorens* were superior over wild types for interspecific crossability was based on only two accessions of *F. vesca* var. *semperflorens* and an undisclosed number of wild accessions of *F. vesca*.

The ability of *F. vesca* to hybridize with four other diploid species validates the hypothesis that *F. vesca* could be a very useful bridge species for the first steps of the synthetic octoploid system. *Fragaria* breeders wishing to use *F. vesca* as a bridge species could use any of these subspecies of *F. vesca* with a high

likelihood of success. Since most *F. vesca* accessions hybridized with most other species in the experiment with the exception of *F. nilgerrensis*, it may be advantageous to focus on *F. vesca* accessions with superior horticultural qualities when introgressing other species. Qualities such as larger fruit size or disease resistance may make an accession more desirable to use, even if only a few seeds were produced in interspecific hybridization. Fadeeva (1966) observed a positive correlation between fruit size of *F. vesca* parental lines and fruit size of interspecific hybrids. Using a well-adapted subspecies would also be an appropriate strategy. If prebreeding were to occur at the diploid level as part of the synthetic octoploid system, this study suggests some variation exists among the accessions and choosing appropriate accessions within subspecies could lead to higher performance levels in the hybrids.

Fragaria vesca has been proposed as a progenitor species within *Fragaria* (Duchesne in Darrow, 1966; Nurnberg-Kruger, 1958; Staudt, 1953). The current study would support this theory due to the crossability between *F. vesca* and the other diploid species tested. Potter et al. (2000) determined that *F. vesca* and *F. nubicola* were the species most closely related to the tetraploid, hexaploid and octoploid *Fragaria* species. *F. iinumae* from China and Japan is believed to be the most ancestral species previously examined (Harrison et al., 1997; Potter et al., 2000). The lack of divergence among the *F. vesca* subspecies in the current study

would suggest it is a younger species (Potter et al., 2000) but it could have been involved in the formation of polyploid species (Bringhurst, 1990; Bringhurst and Senanayake, 1967; Federova, 1946; Staudt, 1959).

The *F. vesca* subspecies demonstrated similar levels of crossability even though they were found in diverse regions. Phylogenetic analysis confirmed the *F. vesca* subspecies (from California, Finland, Nebraska, Sweden, and Washington) were indeed closely related (Potter et al., 2000). *Fragaria vesca*'s adaptation to low light levels enabling it to survive in forest ecosystems (Chabot and Chabot, 1977) may be responsible for its wide distribution throughout the northern continents (Reed, 1966; Staudt, 1989) despite being a young species. *Fragaria vesca* could have migrated during the Miocene (23–5.3 million years ago) with numerous forest species across intercontinental land bridges connecting Asia, North America and Europe (Constance, 1972; Hara, 1972; Li, 1972; Stebbins, 1950). The octoploids *F. virginiana* and *chiloensis* are believed to have arrived in North America via this route (Harrison et al., 1997; Potter et al., 2000). This would explain the morphological similarities between ssp. *vesca* of Europe and ssp. *americana* of eastern North America (Staudt, 1989) and the similar levels of crossability observed in this study. Only those *Fragaria* species adapted to northern latitudes and forest ecosystems could have migrated to North America via northern land bridges.

The crossability results of this study and phylogenetic analysis (Potter et al., 2000) confirm that *F. pentaphylla*, *F. nubicola*, and *F. viridis* are closely related to *F. vesca*. The *F. vesca* × *F. pentaphylla* hybrids have not been previously reported. It was surprising the *F. pentaphylla* had similar crossing results to that of *F. viridis* and *F. nubicola* as this species has unique leaf and fruit morphology suggesting at least some divergence.

The results obtained in this study concur with other research (Evans, 1964; Williams, 1959) which showed that *F. vesca* × *F. nilgerrensis* hybrids are difficult to obtain. To explain these findings, *F. nilgerrensis* is classed as divergent from other diploid species (Harrison et al., 1993, 1997). Earlier studies identified only three *F. vesca* accessions with potential to produce healthy hybrids with *F. nilgerrensis* (Evans, 1964; Williams, 1959; Yarnell, 1931). In this study, 19 *F. vesca* accessions were found that produced hybrid seeds. Seedling stunting (Fadeeva, 1966; Yarnell, 1931) and lethal seedlings (Williams, 1959) have been previously observed in *F. vesca* × *F. nilgerrensis* hybrids. The lack of these phenomena in this study indicates variability in *F. nilgerrensis* and perhaps specific accessions, such as nil1, are superior for interspecific hybridization with *F. vesca*. While it has been suggested that *F. nilgerrensis* cannot be introgressed into the genepool of cultivated strawberries (Hancock et al., 1996), the current study shows encouraging progress toward this goal.

Other differences existed between this and earlier studies. The results of *F. vesca* × *F. nilgerrensis* crosses were particularly noteworthy in this study since very few successful crosses between these two species had been reported previously. If percent seed with embryo is multiplied with percent embryo germination (Table 4), the number of germinating seeds is higher than previous results. For the *F. vesca* × *F. viridis*, *F. vesca* × *F. nubicola*, and *F. vesca* × *F. nilgerrensis* combinations in this study, germination rates were 13%, 5%, and 0% higher, respectively, than earlier studies. While the use of *in vitro* germination did not dramatically increase germination rates compared to traditional methods, it provided additional information regarding the germination of interspecific hybrids. For the combinations of species with low success rates

significant differences among the various combinations of species (Table 4) were due to embryo abortion during seed formation (i.e., seeds with embryos) and not germination rates of fully formed embryos (i.e., embryo germination).

This study was successful in producing many hybrids between *F. vesca* and other diploid species, including the difficult to obtain *F. vesca* × *F. nilgerrensis* hybrids. The lack of significant differences in crossability variables among the four types of *F. vesca* demonstrated that the *F. vesca* subspecies were all acceptable for interspecific hybridization with any of the other four diploid species used in this study and confirms that these species are closely related. Crossability of *F. vesca* indicates that strong potential exists for gene flow between *F. vesca* and other diploid species. Although the number of accessions of other diploid *Fragaria* species was limited, *F. vesca* hybridized with the four other diploid species used in this study, indicating good potential as a bridge species in the synthetic octoploid system. Limitations of germplasm make it impossible to conclude that *F. vesca* is the best bridge species available, and another study (Potter et al., 2000) has shown that *F. nubicola*, like *F. vesca*, is closely related to the octoploid *Fragaria*. As European and North American *F. vesca* subspecies are not divergent enough to differ in interspecific hybridization, it seems more likely that *F. vesca* is a younger rather than a progenitor species. An explanation for the global distribution of *F. vesca* is that specialized physiological adaptations have allowed *F. vesca* to spread in forest ecosystems. This theory provides an alternative to previous theories of *F. vesca* evolution (Duchesne in Darrow, 1966; Longley in Darrow, 1966; Nurnberg-Kruger, 1958; Staudt, 1953).

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