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Pollen Diameter and Guard Cell Length as Predictors of Ploidy in Diverse Rose Cultivars, Species, and Breeding Lines

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ABSTRACT

Roses range from diploid to hendecaploid and determining sporophytic and gametophytic ploidy levels can aid in breeding efforts and genotype and population characterization. Direct chromosome counts require specialized skill and are time consuming. The objectives of this study were to determine the usefulness of pollen diameter and guard cell length to predict sporophytic and gametophytic ploidy levels in a diverse collection of roses (n=428) and demonstrate the utility of pollen size in understanding ploidy transmission in a breeding program. The diameters of 30 pollen grains using acetocarmine staining and the lengths of ten guard cells were recorded per genotype. Sixty-seven roses with reported chromosome counts provided ploidy size ranges from which to predict ploidy of 361 rose genotypes. Root tip squashes were performed to determine actual sporophytic ploidy. Ploidy transmission was documented using breeding lines with known pedigrees and tetraploid female x triploid male crosses to characterize ploidy contribution from triploids. Guard cell length was variable and not useful for generalized ploidy prediction. Pollen diameter accurately predicted 100% of diploid, 91.1% of tetraploid, 80.0% of hexaploid, and 100% of octoploid roses not in or recently derived from section *Caninae* species. Recommended pollen diameter ranges for sporophytic ploidy prediction are: diploid (<35.6 µm), tetraploid (35.6 µm to <43.7 µm), hexaploid (43.7 µm to 47.0 µm), and octoploid (>47.0 µm). Sporophytic ploidy estimation based on pollen diameter was not effective for triploid, pentaploid, and section *Caninae* species and hybrids, although it was useful for gametophytic ploidy estimation. Clones producing $2n$ or $4n$ pollen were identified. Ploidy transmission trends and breeding implications are discussed. Pollen diameter is a fast and useful tool to predict sporophytic and gametophytic ploidy in rose.

Keywords: *Caninae*, flow cytometry, gametophyte, *Rosa*, sporophyte, unreduced gametes
Abbreviations: ANOVA, analysis of variance; **op**, open-pollinated

INTRODUCTION

Roses are among the most economically valuable and longest cultivated of ornamental crops. Major market niches include cut flowers, flowering potted plants, garden or landscape shrubs, essential oil for perfume, and rose hip production for ornamental and culinary uses (Krüssmann 1981; Zlesak 2006). Rose species and complex, interspecific hybrids have long been cultivated for ornamental, medicinal, and culinary use in especially Europe and China (Krüssmann 1981). Exchange of germplasm between Europe and Asia led to hybridization between germplasm groups and resulted in most modern rose cultivars tracing back to a common group of about ten species of European and Asian descent (Gudin 2000; Zlesak 2006).

Roses are native to the Northern hemisphere with a conservative estimate of about 130 species (Zlesak 2006). The genus is divided into three subgenera (Rosa, syn. Eurosa; Hesperhodos; and Platyrhodon); subgenera Rosa represents over 95% of rose species and contains nine sections (*Banksianae*; *Bracteata*; *Caninae*; *Gallicanae*; *Indicae*; *Laevigatae*; *Pimpinellifoliae*; *Rosa*, syn. *Cinnamomeae*; and *Synstylae*) (Krüssmann 1981; Cairns 2000; Joly *et al*. 2006). The basic chromosome number (x) of rose is seven and ploidy level in rose has been documented from diploid to hendecaploid (Zeilinga 1969; Krüssmann 1981; Cairns 2000). Ploidy level can have a profound influence on plant phenotype, physiology, environmental adaptation, pest susceptibility, fertility, and mating success (Levin 2002) and likely contributes to the wide geographical and climatic adaptation of roses. There is a tendency for ploidy level to increase with harsher environmental conditions (Ramsey and Schemske 1998), as seen within the polyploid series comprising the circumpolar rose, *R. acicularis* Lindl. ($2n$=$2x$, $4x$, $6x$, and $8x$) (Lewis 1959; Krüssmann 1981). The combination of these factors makes rose a good model crop for the study and exploitation of ploidy variability.

For some species, like potato and clover, ploidy is closely associated with what pairs of individuals, or even gametes (based on ploidy of gametes), can successfully produce viable offspring (Parrot and Smith 1986; Hanneman 1999). Reproductive limitations imposed in part or whole by ploidy can limit gene flow and can lead to reproductive isolation, even between sympatric populations (Husband and Sabara 2004). Changes in ploidy level, such as meiotic or mitotic polyploidization or haploidization, can overcome reproductive barriers. Although hybrids can be obtained between most rose germplasm groups, incomplete reproductive barriers may be present in rose that favor offspring from the union of gametes having the same ploidy (El Mokadem *et al*. 2001; Leus 2005). Ploidy characterization of individuals and populations can be very useful to better understand population structure, gene flow, and develop effective and efficient breeding strategies.

Direct chromosome counts require individuals with specialized cytological skills and can be a tedious and time consuming process (Ma *et al*. 1996; Zlesak *et al*. 2005). This has led some rose researchers to explore alternative, indirect methods of ploidy assessment including flow cytometry, stomata or guard cell size, and pollen diameter (Semeniuk and Arisumi 1968; Jacob *et al*. 1996; Yokoya *et al*. 2000; Kermani *et al*. 2003; Zlesak *et al*. 2005; Joly *et al*. 2006). Flow cytometry using macerated leaf tissue has become common for sporophytic ploidy characterization in

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Table 1 Examples of pollen diameter ranges (µm) at different sporophytic ploidy levels for six genera.

	Diploid	Triploid	Tetraploid	Hexaploid	Octoploid	Reference
Arachis spp.	33-37	30-60	44-48		51-61	Singsit and Ozias-Akins 1992
Avena spp.	38.0-41.4		39.0-43.9	48.3-49.1	44.8-50.8	Katsiotis and Forsberg 1995
Bromus inermis Leyss			32.6-36.6	34.3-42.2	40.6-45.3	Tan and Dunn 1973
Lilium spp.	67-100	60-113	90-150			McRae 1987
Rosa spp.	Western North American species					
	30.0-39.4		38.3-46.6	45.0-51.6		Erlanson 1931
	Eastern North American section *Rosa* (=*Cinnamomeae*) species					
	23.0-31.3		31.2-36.4	36.4-40.8	37.9-41.8	Lewis 1957
Solanum spp. (wild potato species)	17.3-25.6[a]		24.4-35.1	27.6-28.0		Bamberg and Hanneman 1991

[a] There was one outlying diploid species having a mean pollen diameter of 36.9 µm, *Solanum lycopersicoides* Dun. However, this species is more closely associated with tomato than potato.

recent rose literature (Jacob *et al*. 1996; Yokoya *et al*. 2000; Kermani *et al*. 2003; Leus 2005). However, variability in DNA content among individuals at a particular ploidy level can be great enough to overlap that of another ploidy level and lead to errors in ploidy classification (Jacob *et al*. 1996; Yokoya *et al*. 2000). Variability in DNA content is especially common for complex interspecific hybrids, as most rose cultivars are, due to wide crosses frequently leading to genomic reorganization and alterations in genome size (Levin 2002). It would not be prudent to rely solely on DNA content based on flow cytometry to estimate ploidy when accurate ploidy assessment is imperative.

Pollen diameter can be useful to estimate sporophytic ploidy level in many genera, aiding in species identification, polyploidization studies, and germplasm characterization for breeding and other purposes (Lewis 1957; Semeniuk and Arisumi 1968; Bamberg and Hanneman 1991; Katsiotis and Forsberg 1995; Tenkouano *et al*. 1998; Jacob and Pierret 2000; Zlesak *et al*. 2005). However, pollen diameter ranges at specific ploidy levels can overlap in rose and other crops and lead to uncertainty in ploidy classification of some individuals (**Table 1**) (Erlanson 1931; Lewis 1957; Tan and Dunn 1973; McRae 1987; Bamberg and Hanneman 1991; Singsit and Ozias-Akins 1992; Katsiotis and Forsberg 1995; Tenkouano et al. 1998; Jacob and Pierret 2000). Pollen size as well as pollen morphology are known to vary across genera due to factors including genetic background, chromosome number, pollen maturity, location in the inflorescence, time of pollen grain development during flowering season, temperature, nutrition, and moisture conditions (Stanley and Linskins 1974). In addition, chemical treatments and mounting solutions can affect pollen size, emphasizing the need for consistency when handling samples (Stanley and Linskins 1974).

Although pollen diameter can be variable, it can be a useful predictor of sporophytic ploidy depending on the germplasm and degree of accuracy that is needed. For instance, Bamberg and Hanneman (1991) correctly assessed the sporophytic chromosome number of 76/83 (92%) accessions of potato species. Within this germplasm pollen diameter was not effective in separating tetraploid from hexaploid accessions (all three hexaploid accessions were predicted to be tetraploid and one tetraploid accession was predicted to be hexaploid), but was 93% accurate for separating diploid from tetraploid or hexaploid accessions (Bamberg and Hanneman 1991). Pollen diameter has been proposed as a useful tool for sporophytic ploidy prediction in rose (Erlanson 1931; Lewis 1957; Jacob and Pierret 2000), but its utility has not been well tested. Potential challenges to ploidy prediction in rose include overlap in pollen diameter ranges between sporophytic ploidy levels (Erlanson 1931; Lewis 1957; Jacob and Pierret 2000) and variable ranges reported in the literature which can be attributed in part to variable shape of dry pollen (Erlanson 1931) and different staining treatments.

Pollen diameter has been useful for the identification of male gametophyte ploidy and particularly the identification of parental genotypes which produce $2n$ pollen and the study of the meiotic mutants that govern $2n$ pollen formation (Mok and Peloquin 1975; Watanabe and Peloquin 1989; Zlesak *et al*. 2005; Crespel *et al*. 2006). Rose pollen that is $2n$ is typically ~1.3 times the diameter of n pollen (Crespel *et al*. 2006). Crosses in *Rosa* involving diploid and tetraploid females and a $2n$-pollen producing diploid male (breeding line H3) resulted in variable ploidy levels within six of seven progeny groups, suggesting successful fertilization and seed development from both n and $2n$ pollen (El Mokadem *et al*. 2002a). However, offspring may be skewed towards individuals resulting from the union of gametes possessing the same ploidy (El Mokadem *et al*. 2001). Preference for offspring arising from the union of gametes of the same ploidy is also suggested by a preponderance of tetraploid offspring in crosses between tetraploid and triploid roses (Leus 2005). Pollen diameter, independent of sporophytic chromosome number, can be predictive of reproductive efficiency and the frequency of ploidy level(s) found within progeny.

Roses in the *Caninae* (dog rose) section of *Rosa* are polyploid ($2n$=$4x$, $5x$, or $6x$) (Cairns 2000) and are a classic example of unequal, gender-dependent distribution of chromosomes to gametes (Täckholm 1920; Blackburn and Heslop-Harrison 1921). During typical gametogenesis in *Caninae* species, seven bivalents form and the remaining chromosomes are univalents. The megagametophyte retains a representative of each univalent plus one set of the bivalent pair. The microgametophyte contains one set of the bivalent pair and the univalents are lost, leading to monoploid pollen (n=x=7). Although gametophytic ploidy estimates based on pollen diameter should not be affected, sporophytic ploidy prediction in *Caninae* section species based on pollen diameter is of little value. *Rosa alba* L. ($2n$=$6x$=42) is unique because it has a modified *Caninae* meiosis where the egg is $4x$ and the pollen $2x$ and is suspected to be a natural intersectional cross of a *Caninae* and *Gallicanae* species (Hurst 1925; Atienza *et al*. 2005).

Due to the unique meiosis in section *Caninae*, within species variation is relatively minimal and phenotype of offspring is skewed towards the maternal parent (Kroon and Zeilinga 1974; Nybom *et al*. 1999; Werlemark *et al*. 1999). *Caninae* section species are grown commercially for rose hip production and rootstock (Kroon and Zeilinga 1974; Krüssmann 1981; Buck 1998; Uggla and Nybom 1999). Uniform, seed-propagated lines of *R. canina* L. have been identified and used for rootstock production (Kroon and Zeilinga 1974; Krüssmann 1981). Directed breeding efforts with *Caninae* section species for fruit production is relatively recent and both intrasectional and intersectional hybridization are being explored (Simanek 1982; Uggla and Nybom 1999). *Caninae* section species are generally not within or are far removed in the pedigrees of typical, widely commercialized rose germplasm (Zlesak 2006).

Guard cell or stomatal length is another indirect method useful for ploidy assessment. Guard cell length has been a useful tool for polyploidization studies in rose to characterize and compare the ploidy of the original cultivar and putatively induced polyploids in meristematic layer one (LI) (Semeniuk and Arisumi 1968; Zlesak *et al*. 2005). The *R. carolina* L. complex of North America (species are within section *Rosa*) contains diploid species (*R. blanda* Ait., *R. foliolosa* Nut., *R. nitida* Wild., *R. palustris* Marsh., and *R.*

woodsii Lindl.) and tetraploid species (*R. arkansana* Porter, *R. carolina*, and *R. virginiana* Mill.) thought to be derived from interspecific hybridization between diploid species and polyploidization (Joly *et al*. 2006). Species identification within this complex can be challenging due to introgression and variable morphology. Ploidy level is one diagnostic feature useful for distinguishing species within this complex and guard cell length and pollen diameter do not overlap, except in rare instances, between ploidy levels (Lewis 1957; Joly *et al*. 2006). Variability for guard cell length and its usefulness for general ploidy prediction in rose is largely unexplored.

The objectives of this study are to 1) determine the usefulness of pollen diameter and guard cell length to predict sporophytic and gametophytic ploidy level in a diverse collection of rose cultivars and species and 2) demonstrate the utility of pollen size in understanding ploidy transmission (i.e. $2n$/$4n$ pollen production, *Caninae* section meiosis, ploidy of breeding lines from parents characterized for ploidy, and contributions from triploids) in a rose breeding program.

MATERIALS AND METHODS

Plant material

Rose species, cultivars and germplasm releases, and breeding lines (44, 214, and 170 genotypes, respectively) were used for this study and represent a wide germplasm base (i.e. species from seven sections of *Rosa*, cultivars from 24 commercial/horticultural classes, and diverse breeding lines including descendants of North American species and intersectional crosses with *Caninae* species). Roses were grown at the University of Minnesota Landscape Arboretum, University of Minnesota St. Paul greenhouses, Linder's Garden Center (St. Paul, Minnesota), Sam Kedem Nursery (Hastings, Minnesota), and the author's rose gardens at River Falls and Monroe Center, Wisconsin and St. Paul, Minnesota. A limited number of leaf, pollen, and root tip samples were also contributed via post from private rose growers. Data were collected over ten years (1999-2008).

Pollen and guard cell measurements

Approximately one day prior to anthesis, rose anthers were collected and bulked from at least two flowers and allowed to dehisce in the laboratory at room temperature in open topped, plastic canisters made to hold 35-mm film. Rose pollen was stained with acetocarmine (≥1 min) and viewed at 400X magnification using a light microscope. Transfer of pollen to a drop of acetocarmine stain on a microscope slide was accomplished by using the tip of a wooden toothpick moistened in acetocarmine and rubbed among dried anthers. After transfer of pollen, a cover slip was placed over the drop. The diameters of 30 well-stained pollen grains per genotype were recorded in one of two ways: using a calibrated optical eyepiece graticule or determining measurements from digital images using Image Pro® 4.1 (Media Cybernetics®, Silver Spring, MD) calibrated with the same 40X objective lens used for photo acquisition using the software Spot RT 3.0 (Diagnostic Instruments, Inc., Sterling Heights, MI).

Guard cell length was measured using epidermal imprints due to difficulty in obtaining epidermal peels in rose. Two fully-expanded terminal leaflets per genotype were pressed (abaxial side down) into a drop of fast-drying glue (Kwik fix® Super glue plus™, Chemence, Inc., Alpharetta, GA) on a glass microscope slide and removed soon after glue hardened. A drop of acetocarmine and a cover slip were placed over the imprint for greater contrast during examination. The lengths of five guard cell imprints (one guard cell measured per stomatal pair) were recorded per leaflet (ten measurements/genotype), and length was measured using the same methods and magnification described for measure-ment of pollen diameter.

Ploidy prediction

Pollen diameter and guard cell length were determined for 67 rose cultivars and species genotypes with previously reported ploidy level via direct chromosome counts (before ploidy estimation by flow cytometry became routine) in order to establish ranges for these traits at each represented ploidy level (**Table 2**). These roses represent a wide diversity within *Rosa* (16 horticultural classes) and were selected in part based on availability to the author. The ranges for pollen diameter and guard cell length were the basis by which sporophytic ploidy predictions were made for roses where direct sporophytic ploidy assessment was not yet reported. If the ranges in pollen diameter between diploid, tetraploid, and hexaploid genotypes did not overlap or meet, the midpoint between ranges was used as a cut off between ploidy levels for ploidy prediction. Special consideration was given to pollen diameter of rose species within the section *Caninae* due to the expectation of one set of chromosomes (n=x=7) in the pollen nuclei. For pollen analysis these roses were grouped with diploids because pollen of both groups are expected to have the same gametophytic ploidy level and be comparable in size. In addition, *R. alba* ($2n$=$6x$=42) is a suspected intersectional *Caninae* section hybrid that has a modified meiosis where the egg is $4x$ and the pollen $2x$ (Hurst 1925). For pollen analysis *R. alba* cultivars were grouped with pollen of non *Caninae* section tetraploids.

In the process of pollen measurement, some clones were found that possessed a relatively high proportion (>5%) of distinctly larger pollen. Thirty pollen grains of both n pollen and large pollen were measured for these genotypes. Depending on the relative size of large pollen to n pollen, large pollen was classified as either $2n$ or $4n$ (**Fig. 1**). The diameter of $2n$ pollen in rose is ~1.3X the diameter of n pollen (Crespel *et al*. 2006). Little has been re-

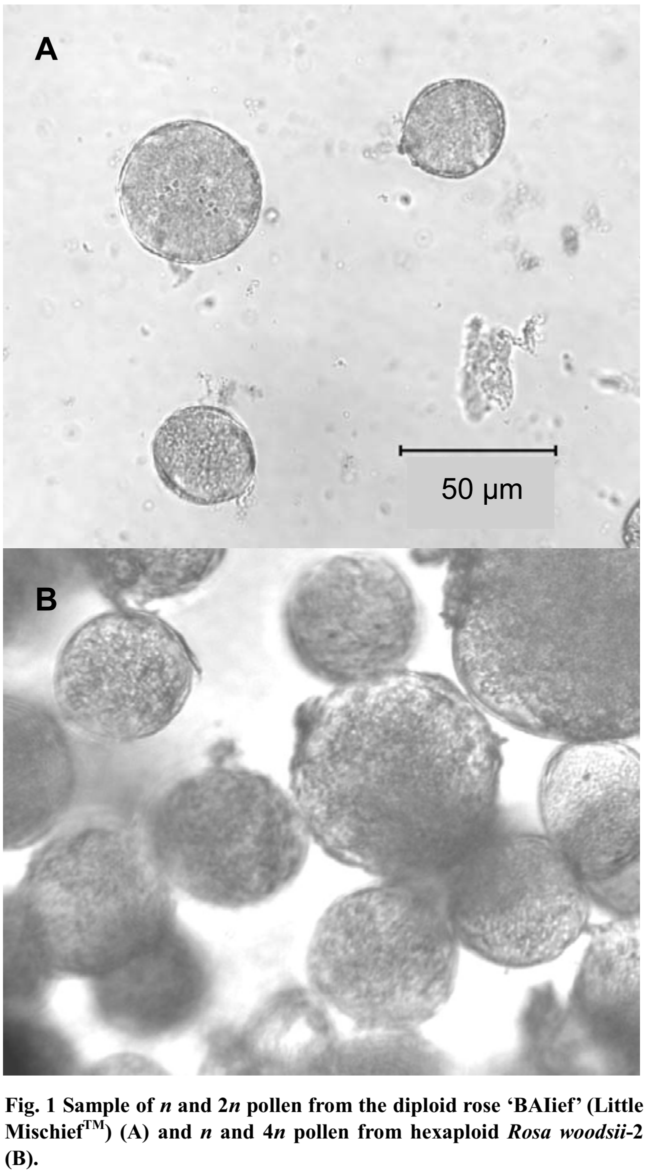

Fig. 1 Sample of n and $2n$ pollen from the diploid rose 'BAIief' (Little Mischief™) (A) and n and $4n$ pollen from hexaploid *Rosa woodsii*-2 (B).

ported for clones which produce 4*n* pollen. Bamberg and Hanneman (1991) describe a potato accession with 4*n* pollen and the diameter of 4*n* pollen was ~1.8*x* the diameter of *n* pollen.

Direct ploidy assessment

Root tip squashes were used to directly count chromosomes and determine ploidy for genotypes where ploidy level had not been reported and where published ploidy came into question. Chromosomes of n≥5 metaphase cells were observed per genotype. Actively growing root tips were harvested and stored in vials of water on ice for 24 h. Root tips were subsequently fixed in Farmer's fixative (3:1 (v/v), 95% ethanol: glacial acetic acid) and refrigerated until observation. Root tips were hydrolyzed in 6 N HCl for 90 min at room temperature just prior to squashing and acetocarmine was used for staining. In rare instances when potted plants growing on their own roots (not grafted) were not available and stem cuttings did not produce adventitious roots for examination, shoot apical meristems were used to obtain mitotic cells (i.e. *R. rubiginosa* L. and some *R. rubiginosa* hybrids). Shoot tip squashes were performed using the same protocol described for root tip squashes.

Exploring trends for indirect chromosome measurements based on ploidy and section

Actual and predicted mean pollen diameters for each pollen ploidy level were correlated. Pollen from diploids and *Caninae* section species were classified as 1*x* pollen; tetraploid and alba roses, 2*x* pollen; hexaploids, 3*x* pollen; 2*n* pollen from tetraploids, 4*x* pollen; 4*n* pollen from tetraploids, 8*x* pollen; and 4*n* pollen from a hexaploid, 12*x* pollen. Triploid and pentaploid genotypes (not from the *Caninae* section) and *Caninae* section rose species genotypes or hybrids with unexpectedly large pollen were omitted from this analysis because of ambiguity of gametophytic ploidy level. Expected pollen diameter calculations rely on the assumption that pollen volume is proportional to ploidy level. The mean actual diameter of 1*x* pollen was used to calculate the expected diameters for the other pollen ploidy levels. Expected pollen diameters were calculated by multiplying the mean actual diameter of 1*x* pollen by the cubed root of male gametophytic ploidy (mgp(*x*)) (Bamberg and Hanneman 1991).

$$\text{Expected pollen diameter for pollen of ploidy } x = \bar{x}\ \sqrt[3]{mgp(x)}$$

Pollen diameter and guard cell length of rose species grouped by section were compared. Pollen diameter across gametophytic ploidy levels was standardized for easier comparisons in the table. However, actual pollen diameter was used for the analysis of variance (ANOVA) with ploidy as a covariate. Standardization of pollen diameter was calculated by taking the mean observed pollen diameter divided by the cubed root of pollen ploidy and multiplied by the ratio of predicted diameter (*pdia*) over observed diameter (*odia*) for the particular pollen ploidy class as previously calculated.

$$\text{Standardization of pollen diameter} = \frac{\bar{x}}{\sqrt[3]{mgp(x)}}\left(\frac{pdia}{odia}\right)$$

If multiple genotypes of a species were recorded, the species mean was used for analysis within a section so a particular species was not disproportionately represented.

Ploidy transmission from triploid males

Crosses were made between tetraploid female parents ('BUCbi', 1A10, 4A29, 1B30, and 1990-1) and triploid males ('KORbin', 1G84, 2G102, 1B43, and 1990-6) in order to survey the ploidy level(s) found among progeny and better understand the ploidy contribution of triploid males to progeny. Variability in ploidy is assumed to come from the triploid males rather than the tetraploid female parents. Typical emasculation and pollination techniques were used to perform crosses, and cold stratification (4°C, 10 weeks) of achenes was used to promote germination. Direct ploidy determination of progeny was performed using root tip squashes as previously described. Additionally, self-fertilized seedlings (parent plant was isolated during flowering) were raised (n>30) of *R. pomifera* Herrmann-3, a triploid derived via poly-embryony. A sample (n=5) of *R. pomifera*-3 seedlings were confirmed for ploidy in order to infer if *Caninae* section meiosis had been altered and the ploidy of female and male gametophytes.

Statistical analysis

Analysis of variance was used to assess the influence of section on guard cell length and actual pollen diameter. The two fixed factors in the model were section and species (nested in section); genotype (nested in species) was designated in the model as a random factor. Sporophytic ploidy level was used as a covariate in the analyses. A modification was made for pollen diameter of section *Caninae* roses being designated as diploid because their pollen would be monoploid (like that of diploids) due to their unique meiosis. Sections represented by only one genotype were removed from the analysis due to no replication for section. Pearson's correlation was calculated for expected and observed pollen diameters over gametophytic ploidy levels. Kendall's Tau correlation was calculated for sporophytic ploidy level and guard cell length due to ploidy being a categorical variable. Statistical analyses were performed using SPSS software (version 13.0 for Windows; SPSS, Chicago, Ill.).

RESULTS

Mean pollen diameter ranges across roses with previously reported direct sporophytic chromosome counts were: diploid (26.2-35.9 μM), tetraploid (35.9-42.8 μM), and hexaploid (43.9-44.4 μM) (**Table 2**). Pollen diameter ranges were distinct with the exception of the upper and lower ranges of diploid and tetraploid roses meeting at 35.9 μM. The range for triploid (34.6-40.4 μM) roses overlapped that of diploids and tetraploids (**Table 2**); therefore, gametophytic ploidy of such pollen was uncertain and prediction of roses being triploid based on pollen diameter did not occur. Pollen diameter of 'Betty Bland' was unusually large (39.7 μM) compared to the other roses reported to be diploid (26.2-35.9 μM). Therefore, the ploidy of 'Betty Bland' was confirmed with a root tip squash and it was triploid. The mean diameter of 2*n* pollen for the tetraploid cultivar MEIhelvet (48.3 μM) was used to estimate the diameter of *n* pollen of octoploids (n=4x=28). Guard cell length varied greatly and ranges overlapped across all ploidy levels: diploids (14.3-26.6 μm), triploids (20.3-32.5 μm), tetraploids (21.7-32.9 μm), and hexaploids (25.3-26.8 μm). The Kendall's Tau correlation coefficient between sporophytic ploidy and guard cell length was positive for all 428 roses assessed (r=0.26; P<0.001), but quite low. Ranges used for estimating sporophytic ploidy level relied solely on pollen diameter and were: diploid (<35.9 μm), tetraploid (≥35.9-43.4 μm), hexaploid (>43.4-46.4 μm), and octoploid (>46.4 μm) roses.

Ploidy predictions were made for cultivars, species, and breeding lines (**Tables 3, 4**) based on pollen diameter. Out of the 354 roses where ploidy was predicted (*Caninae* section species and their hybrids were omitted, except for alba cultivars), all 73 confirmed diploids were predicted to be diploid, 91.1% confirmed tetraploids (164/180) were predicted to be tetraploid (2.8% were predicted to be diploid and 6.1% hexaploid), and 80.0% confirmed hexaploids (4/5) were predicted to be hexaploid (one was predicted to be octoploid) (**Table 5**). Of the triploids, 16.3% were predicted to be diploid (16/98), 70.4% tetraploid (69/98), 9.2% hexaploid (9/98), and 4.1% octoploid (4/98). The one pentaploid (not of *Caninae* section origin) was predicted to be tetraploid. Overall, for confirmed diploid, tetraploid, and hexaploid roses (roses with even sets of chromosomes and not of *Caninae* section origin) there was 93.4% accuracy (241/258) in sporophytic ploidy prediction based on mean pollen diameter.

Considering the pollen diameter of the 67 roses with reported chromosome counts together with the additional 354 roses (direct chromosome counts made in this study and

Table 2 Pollen diameter, guard cell length, and horticultural class for rose cultivars and species grouped by reported sporophytic ploidy.

		Horticultural	**Gametophytic**	**Mean ± SD (µm)**	
	Cultivar[a]	**class**[b]	**ploidy (*x*)**	**Pollen**	**Guard cell**
Diploid[c]	95-1[9]	14	1	34.2 ± 1.7	20.8 ± 1.6
	95-2[9]	14	1	34.7 ± 1.7	19.1 ± 1.2
	Ballerina[8]	6	1	32.0 ± 1.8	23.2 ± 2.0
	Belle Poitevine[1]	8	1	33.6 ± 1.7	14.3 ± 2.5
	Blanc double de Coubert[1]	8	1	35.2 ± 1.6	16.1 ± 1.8
	Cantabrigiensis[1]	15	1	35.8 ± 3.3	18.4 ± 0.7
	Frau Dagmar Hastrup[1]	8	1	34.5 ± 1.7	19.6 ± 0.7
	Grootendorst Supreme[7]	8	1	34.8 ± 1.6	19.0 ± 0.8
	Hansa[7]	8	1	30.8 ± 1.6	12.3 ± 0.7
	Katharina Ziemet[7]	14	1	30.0 ± 1.6	25.3 ± 3.3
	Madame Georges Bruant[1]	8	1	31.2 ± 3.2	20.0 ± 2.0
	Max Graf[1]	8	1	31.3 ± 2.7	17.5 ± 1.0
	Nastarana[1]	13	1	32.8 ± 1.9	25.9 ± 1.6
	Rosa amblyotis Meyer[1]	16	1	34.2 ± 2.0	18.6 ± 1.1
	R. banksiae lutea Rehder[1]	16	1	26.2 ± 1.4	19.4 ± 2.1
	R. foliolosa Nutt.[1]	16	1	32.8 ± 1.6	26.2 ± 2.2
	R. hugonis Hemsl.[1]	16	1	35.7 ± 2.9	22.5 ± 1.9
	R. macounii Greene[1]	16	1	33.3 ± 1.5	20.3 ± 2.6
	R. maximowicziana Regel[1]	16	1	33.2 ± 1.6	18.4 ± 1.8
	R. nitida Wild[1]	16	1	34.9 ± 2.6	21.0 ± 1.6
	R. primula Boulenger[1]	16	1	34.5 ± 2.4	19.7 ± 1.8
	R. roxburghii Tratt.[1]	16	1	32.6 ± 2.1	21.4 ± 2.3
	R. setigera Michx.[1]	16	1	34.9 ± 2.1	26.6 ± 3.2
	R. wichurana Crépin[1]	16	1	34.5 ± 2.0	24.4 ± 3.6
	R. woodsii Lindl.[1]	16	1	35.9 ± 1.9	22.8 ± 1.9
	Robin Hood[1]	6	1	32.4 ± 1.7	17.9 ± 1.7
	Sarah van Fleet[1]	8	1	34.2 ± 2.1	22.3 ± 3.9
	Schneezwerg[1]	8	1	31.6 ± 1.1	15.2 ± 1.3
	Sir Thomas Lipton[7]	8	1	32.3 ± 2.7	25.0 ± 2.1
	Thérèse Bugnet[1]	8	1	31.2 ± 1.3	21.5 ± 1.3
	White Pet[1]	14	1	32.3 ± 3.2	25.5 ± 3.1
	Yvonne Rabier[1]	14	1	32.4 ± 1.2	17.9 ± 1.1
	Average		**1**	**33.1 ± 2.0**	**20.6 ± 3.6**
Triploid	Betty Bland[1d]	15	unknown	39.7 ± 3.5	29.6 ± 3.0
	Fimbriata[4]	8	unknown	35.1 ± 5.4	20.3 ± 2.2
	Irene of Denmark[1]	2	unknown	38.0 ± 3.9	32.5 ± 2.7
	La France[4]	10	unknown	34.6 ± 4.1	28.2 ± 1.9
	Rose à Parfum de l'Hay[7]	8	unknown	40.4 ± 6.3	23.5 ± 1.3
	Average			**37.6 ± 2.6**	**26.8 ± 4.9**
Tetraploid *Caninae* section species	*R. glauca* Pouret-1[1e]	16	1	33.5 ± 1.9	26.9 ± 1.3
	R. glauca-2[1]	16	1	35.0 ± 2.0	27.1 ± 1.9
	R. mollis Smith[1]	16	1	33.8 ± 2.1	22.9 ± 1.9
	R. pomifera Herrmann-1[1]	16	1	32.8 ± 2.0	22.8 ± 1.3
	R. pomifera-2[1]	16	1	32.8 ± 1.9	22.6 ± 2.1
	Average		**1**	**33.6 ± 0.9**	**24.4 ± 2.3**
Tetraploid	Autumn Damask[1]	1	2	40.1 ± 3.0	21.7 ± 1.1
	Basye's Blueberry[2]	15	2	39.6 ± 2.6	31.5 ± 2.4
	Conrad Ferdinand Meyer[1]	8	2	42.8 ± 2.6	26.4 ± 2.1
	Dupontii[1]	11	2	40.9 ± 2.2	29.5 ± 1.4
	Frau Karl Druschki[1]	7	2	38.3 ± 2.8	37.0 ± 2.2
	Frühlingsgold[1]	9	2	41.0 ± 2.4	29.5 ± 2.6
	Harison's Yellow[1]	4	2	38.4 ± 3.1	31.1 ± 1.5
	Marguerite Hilling[1]	5	2	40.9 ± 3.6	24.1 ± 3.8
	MEIhelvet (Sonia)[3]	3	2	40.4 ± 2.0	32.9 ± 3.7
		2*n* pollen	4	48.3 ± 1.9	
	Nevada[1]	5	2	40.1 ± 3.6	33.7 ± 4.3
	Peace[5]	10	2	39.6 ± 2.0	27.9 ± 2.3
	Quatre Saisons Blanc Mousseux[1]	12	2	37.7 ± 2.7	22.9 ± 2.2
	Queen Elizabeth[6]	3	2	40.1 ± 2.2	30.3 ± 3.1
	R. foetida bicolor (Jacquin) Willmott[1]	16	2	38.7 ± 2.9	25.4 ± 2.8
	R. foetida persiana Rehder[1]	16	2	39.5 ± 4.3	23.5 ± 2.0
	R. laxa Retzius[1]	16	2	39.8 ± 2.9	28.0 ± 2.2
	R. gallica versicolor L.[1]	16	2	40.7 ± 1.7	24.7 ± 2.0
	R. pendulina L.[1]	16	2	39.7 ± 1.9	27.1 ± 1.8
	R. spinosissima altaica Bean[1]	16	2	40.1 ± 3.3	31.4 ± 2.7
	R. virginiana Mill.-1[1]	16	2	35.9 ± 1.8	26.0 ± 1.9
	R. virginiana-2[1]	16	2	37.4 ± 1.4	24.5 ± 2.1
	Stanwell Perpetual[1]	9	2	39.8 ± 2.3	23.6 ± 2.1
	York and Lancaster[1]	1	2	38.8 ± 2.8	24.9 ± 1.6
	Average (without 2*n* pollen)		**2**	**39.6 ± 1.4**	**27.7 ± 4.0**
Hexaploid	*R. nutkana* Presl-1[1]	16	3	43.9 ± 2.5	25.3 ± 1.5
	R. nutkana-2[1]	16	3	44.4 ± 2.2	26.8 ± 2.7
	Average		**3**	**44.2 ± 0.4**	**26.1 ± 1.1**

[a] Cultivar name is followed by trademark or exhibition name, if different, in parenthesis.
[b] 1 Damask; 2 Floribunda or climbing floribunda; 3 Grandiflora; 4 Hybrid foetida; 5 Hybrid moyesii; 6 Hybrid musk; 7 Hybrid perpetual; 8 Hybrid rugosa; 9 Hybrid spinosissima; 10 Hybrid tea or climbing hybrid tea; 11 Miscellaneous old garden rose; 12 Moss; 13 Noisette; 14 Polyantha; 15 Shrub; 16 Species.
[c] Reported sporophytic ploidy; 1 Cairns 2000; 2 Ma *et al.* 2000; 3 Meynet *et al.* 1994; 4 Rowley 1960b; 5 Shahare and Shastry 1963; 6 Svejda 1979; 7 Walker and Hunter 1954; 8 Yokoya *et al.* 2000; 9 Zlesak *et al.* 2005.
[d] 'Betty Bland' is reported to be diploid. A root tip squash was conducted due to its unusually large pollen and it was found to be triploid.
[e] Numbers (i.e. -1 and -2) following species indicate different clones.

Table 3 Pollen diameter, guard cell length, ploidy predictions, and horticultural class for rose cultivars and species grouped by confirmed sporophytic ploidy.

	Cultivar[a]	Horticultural class[b]	Predicted ploidy (*x*)		Mean ± SD (µm)	
			Gametophyte	Sporophyte	Pollen	Guard cell
Diploid	Aylsham	9	1	2	32.3 ± 1.0	19.7 ± 1.6
	Baby Faurax	18	1	2	33.0 ± 1.7	23.7 ± 1.6
	BAIief (Little Mischief)	19	1	2	33.0 ± 1.5	30.6 ± 2.2
		2*n* pollen	2		39.6 ± 2.1	
	BAIpome (Pink Gnome)	19	1	2	35.0 ± 2.4	29.8 ± 3.2
	Corylus	19	1	2	32.6 ± 1.5	25.1 ± 4.5
	Elmshorn	19	1	2	34.3 ± 3.6	32.2 ± 2.8
	JACcasp (Happy Trails)	15	1	2	34.4 ± 2.5	33.4 ± 2.2
	Lillian Gibson	19	1	2	33.0 ± 2.7	23.2 ± 2.0
	Marie Pavié	18	1	2	32.5 ± 1.7	19.3 ± 1.5
	Martin Frobisher	10	1	2	32.7 ± 2.1	15.7 ± 1.9
	MEIflopan (Alba Meidiland)	19	1	2	30.0 ± 1.6	26.6 ± 2.3
	Mevrouw Nathalie Nypels	18	1	2	30.9 ± 1.8	30.7 ± 3.0
	MORcheri (Sweet Chariot)	15	1	2	33.3 ± 2.1	22.4 ± 2.2
	MORyelrug (Topaz Jewel)	10	1	2	31.9 ± 1.8	31.6 ± 2.3
		2*n* pollen	2		39.3 ± 2.8	
	Polstjärnan	14	1	2	28.5 ± 2.3	28.2 ± 2.1
	POUlans (Martha's Vineyard)	19	1	2	30.8 ± 2.0	32.7 ± 1.8
	POUlemb (Cliffs of Dover)	19	1	2	32.4 ± 1.4	25.7 ± 1.9
	POUlrijk (Madison)	19	1	2	32.9 ± 1.8	29.1 ± 2.7
	POUlrust (Cambridge)	19	1	2	32.5 ± 1.7	33.9 ± 3.8
	POUltumb (Tumbling Waters)	19	1	2	32.2 ± 2.4	24.3 ± 2.4
	Rosa blanda Aiton-1[c]	20	1	2	32.9 ± 1.7	22.5 ± 2.3
	R. blanda-2	20	1	2	31.5 ± 1.6	18.5 ± 1.8
	R. blanda-3	20	1	2	29.4 ± 1.6	16.8 ± 1.1
	R. carolina L.	20	1	2	34.2 ± 1.8	22.9 ± 1.2
	R. cinnamomea L.	20	1	2	32.7 ± 2.3	15.0 ± 1.1
	Renae	2	1	2	35.3 ± 2.0	21.0 ± 1.3
	SPEvu (Lovely Fairy)	18	1	2	32.7 ± 1.9	27.0 ± 1.9
	The Fairy	18	1	2	33.1 ± 2.1	23.2 ± 2.3
	WEOpop (Gourmet Popcorn)	15	1	2	33.5 ± 2.2	22.2 ± 2.4
Triploid	ANGelsie (Lady Elsie May[c])	19	2	4	37.1 ± 4.3	31.7 ± 3.7
	Arts Rose	19	2	4	38.0 ± 4.1	23.0 ± 0.9
	Awakening	14	1	2	35.5 ± 2.7	35.9 ± 2.0
	BAIeam (Day Dream[d])	19	2	4	40.5 ± 3.7	36.3 ± 3.1
	BAIfairy (Mystic Fairy)	19	2	4	39.1 ± 5.9	32.5 ± 2.3
	BAIngo (Last Tango)	19	2	4	42.3 ± 3.9	35.0 ± 2.1
	BAIoon (Tahitian Moon)	19	2	4	40.2 ± 4.8	38.3 ± 2.6
	BAIore (Polar Joy)	19	2	4	39.1 ± 8.3	28.5 ± 3.7
	BAIset (Sunrise Sunset)	19	2	4	37.2 ± 5.0	28.2 ± 2.0
	Belinda's Dream	19	2	4	36.4 ± 4.4	34.3 ± 2.8
	BRIincog (Incognito)	15	3	6	43.5 ± 3.0	33.9 ± 3.5
	Crimson Shower	13	2	4	36.1 ± 2.4	34.6 ± 2.8
	DEVrudi (First Light[d])	19	2	4	38.8 ± 2.4	31.1 ± 1.9
	Dr. Huey	14	2	4	39.4 ± 3.9	37.9 ± 4.9
	Erfurt	8	1	2	30.8 ± 2.1	32.2 ± 3.6
	Flower Carpet Appleblossom	19	1	2	34.8 ± 6.6	34.8 ± 3.5
	Flower Carpet Yellow	19	2	4	42.9 ± 5.7	35.6 ± 1.9
	Hi, Neighbor	3	2	4	40.3 ± 2.5	37.1 ± 2.7
	INTerfire (Orange Fire)	2	2	4	39.9 ± 4.2	34.4 ± 4.3
	INTerlav (Lavender Dream)	19	2	4	36.4 ± 3.4	34.5 ± 4.1
	Jeanne Lajoie	15	2	4	38.0 ± 2.6	34.2 ± 3.7
	John Davis	7	2	4	39.5 ± 2.0	39.3 ± 2.1
	JP Connell	19	3	6	43.5 ± 4.0	38.4 ± 3.5
	Karl Förster	11	2	4	41.2 ± 4.8	31.3 ± 3.4
	KORbin (Iceberg)	2	2	4	42.0 ± 6.9	28.6 ± 2.5
	KORgosa (Robusta)	19	3	6	44.0 ± 4.3	30.9 ± 3.3
	KORtemma (Red Ribbons)	19	2	4	39.7 ± 3.5	29.3 ± 2.2
	Léonie Lamesch	18	4	8	50.7 ± 4.2	37.7 ± 2.8
	Lila Banks	19	2	4	43.1 ± 4.1	33.6 ± 2.3
	MEIcoublan (White Meidiland)	19	2	4	37.9 ± 5.5	27.5 ± 1.5
	MEIdomonac (Bonica[d])	19	2	4	42.4 ± 2.5	34.7 ± 1.9
	MEIgali (Starina)	15	2	4	38.6 ± 5.0	36.5 ± 2.9
	MEIkrotel (Scarlet Meidiland)	19	2	4	36.7 ± 2.3	35.6 ± 3.4
	MEImodac (Royal Bonica)	19	2	4	41.3 ± 3.2	37.5 ± 2.9
	MEIneble (Red Meidiland)	19	1	2	32.2 ± 3.5	34.0 ± 4.2
	MEIpelta (Fuschia Meidiland)	19	2	4	37.4 ± 4.2	35.1 ± 3.9
	MEIpotal (Carefree Delight[d])	19	1	2	34.8 ± 2.7	33.8 ± 3.7
	MEIrumour (Cherry Meidiland)	19	2	4	36.2 ± 2.9	31.2 ± 2.2
	MORnine (Roses are Red)	19	2	4	41.2 ± 3.4	31.0 ± 5.2

Table 3 (Cont.)

	Cultivar[a]	Horticultural class[b]	Predicted ploidy (x)		Mean ± SD (μm)	
			Gametophyte	Sporophyte	Pollen	Guard cell
Triploid	MORtange (Tangerine Jewel)	4	4	8	48.5 ± 4.2	28.2 ± 2.0
	MORten (Linda Campbell)	10	2	4	39.6 ± 3.8	29.9 ± 2.0
	MORyears (Out of Yesteryear)	19	3	6	43.6 ± 4.5	36.2 ± 2.3
	Nearly Wild	2	1	2	33.8 ± 3.8	19.0 ± 1.4
	NOAla (Flower Carpet Coral)	19	1	2	35.1 ± 2.1	35.1 ± 3.3
	NOAtraum (Flower Carpet Pink)	19	1	2	35.4 ± 3.0	33.2 ± 2.8
	ORAwichkay (Starry Night[d])	19	2	4	37.9 ± 4.5	34.9 ± 3.7
	POUlgode (Lexington)	19	2	4	43.0 ± 3.8	40.6 ± 4.5
	POUlor (Mystic)	19	1	2	34.8 ± 3.8	28.6 ± 2.5
	Prairie Harvest	19	4	8	51.8 ± 5.6	30.5 ± 2.8
	PRObil (Brilliant Pink Iceberg)	2	2	4	39.5 ± 3.7	34.7 ± 2.4
	R. carolina plena	20	1	2	35.7 ± 2.5	28.2 ± 1.8
	R. pomifera Herrmann-3	20	1		32.8 ± 1.5	21.4 ± 1.6
	RADcon (Pink Knock Out)	19	3	6	45.5 ± 4.2	31.4 ± 3.6
	RADcor (Rainbow Knock Out[d])	19	2	4	39.6 ± 3.3	31.1 ± 3.5
	RADrazz (Knock Out[d])	19	4	8	46.8 ± 5.2	34.6 ± 2.9
	RADyod (Blushing Knock Out)	19	3	6	43.8 ± 3.7	33.2 ± 3.4
	Red Cascade	15	2	4	35.9 ± 3.0	33.1 ± 3.3
	RIPriver (Riverbanks)	19	2	4	40.2 ± 3.7	39.3 ± 3.8
	Sea Foam	19	1	2	31.1 ± 2.4	29.4 ± 3.2
	Simon Fraser	19	2	4	37.1 ± 2.8	33.4 ± 2.2
	TANorstar (Tropicana[d])	12	2	4	38.1 ± 3.7	40.7 ± 2.8
	WEKboroco (Rockin' Robin)	19	2	4	42.9 ± 3.5	36.3 ± 2.4
	WEKcisbako (Home Run)	19	2	4	42.0 ± 4.3	34.5 ± 2.7
	WEKemilcho (Neon Cowboy)	15	2	4	41.8 ± 4.1	39.9 ± 2.8
	White Dawn	14	2	4	39.5 ± 4.2	33.9 ± 2.5
	WILspreader (Scarlet Spreader)	19	2	4	39.9 ± 3.5	44.3 ± 2.6
	ZLEhanruby (Hannah Ruby)	15	2	4	38.5 ± 3.7	33.4 ± 1.7
Tetraploid	Alika	6	2	4	38.2 ± 2.5	23.4 ± 1.8
	Applejack	19	2	4	36.6 ± 2.5	35.7 ± 4.4
	AROsnap (Gingersnap)	2	2	4	43.3 ± 2.6	31.0 ± 1.8
	AUSbells (Bow Bells)	19	2	4	38.9 ± 2.6	39.3 ± 4.1
	AUSblush (Heritage)	19	2	4	41.2 ± 2.8	34.3 ± 3.3
	AUSclough (Sir Clough)	19	2	4	40.7 ± 2.8	27.3 ± 1.8
	BAIall (Great Wall)	19	2	4	42.2 ± 3.9	30.3 ± 2.3
	BAIcer (Island Dancer)	19	2	4	40.8 ± 2.1	34.6 ± 3.3
	BAIcker (Firecracker)	19	2	4	41.0 ± 2.9	33.2 ± 3.3
	BAIcream (Macy's Pride)	19	3	6	44.6 ± 3.3	34.9 ± 3.2
	BAIeye (Golden Eye)	19	3	6	44.5 ± 3.1	38.0 ± 3.7
	BAIface (Funny Face)	19	2	4	42.8 ± 3.1	32.0 ± 2.0
	BAIhero (My Hero)	19	2	4	40.5 ± 3.1	34.3 ± 2.2
	BAIine (Yellow Submarine)	19	2	4	39.0 ± 2.9	36.9 ± 3.9
	BAIkye (Sierra Skye)	19	2	4	40.4 ± 1.9	34.5 ± 2.3
		$2n$ pollen	4		48.6 ± 1.8	
	BAInder (Hot Wonder)	19	2	4	41.9 ± 2.4	35.2 ± 2.3
	BAIngo (Grandma's Blessing)	19	2	4	42.3 ± 4.3	33.4 ± 3.5
	BAIoist (Orange Impressionist)	19	2	4	39.9 ± 2.6	33.0 ± 1.4
	BAIpeace (Love and Peace[d])	12	2	4	40.1 ± 3.5	38.3 ± 3.2
	BAIsist (Salmon Impressionist)	19	2	4	40.2 ± 2.2	29.8 ± 3.0
		$2n$ pollen	4		50.7 ± 3.7	
	BAIsme (Kiss Me)	19	2	4	41.7 ± 2.6	31.1 ± 2.0
	BENmfig (Jilly Jewel)	15	2	4	41.0 ± 2.4	30.0 ± 2.2
	BRIdoris (Doris Morgan)	15	3	6	43.7 ± 3.6	30.4 ± 2.9
	BUCbi (Carefree Beauty)	19	2	4	41.3 ± 1.6	20.7 ± 1.3
	Champlain	19	2	4	41.9 ± 2.3	26.9 ± 1.8
	Chorale	19	2	4	38.2 ± 1.9	37.4 ± 5.1
	Chuckles	2	2	4	40.4 ± 2.6	25.4 ± 1.8
	Como Park	19	2	4	43.3 ± 3.8	32.9 ± 2.7
	Complicata	6	2	4	40.3 ± 3.5	26.9 ± 1.6
	Daksong (Dakota Song)	19	2	4	37.4 ± 3.9	36.6 ± 3.5
	Daksun (Dakota Sun)	19	2	4	38.3 ± 3.4	41.2 ± 4.3
	De Montraville	19	2	4	38.9 ± 3.2	34.7 ± 4.2
	DELmur (Altissimo)	14	2	4	39.8 ± 2.3	40.5 ± 2.0
	Dorcas	19	2	4	39.4 ± 2.4	26.9 ± 2.3
	Folksinger	19	2	4	40.1 ± 3.4	34.9 ± 2.1
	Freckles	19	2	4	42.3 ± 3.7	34.2 ± 2.4
	Frontenac	19	2	4	40.0 ± 2.8	27.7 ± 1.9
		unknown n pollen	unknown		73.1 ± 6.6	
	Frühlingsduft	11	2	4	38.7 ± 1.8	24.8 ± 2.3
	Golden Wings	19	2	4	40.9 ± 4.6	33.7 ± 2.2
	Haidee	19	2	4	37.6 ± 1.8	26.6 ± 3.0

Table 3 (Cont.)

	Cultivar[a]	Horticultural class[a]	Predicted ploidy (x)		Mean ± SD (µm)	
			Gametophyte	Sporophyte	Pollen	Guard cell
Tetraploid	Hawkeye Belle	19	2	4	41.4 ± 1.9	36.2 ± 3.9
	Henri Martin	17	2	4	40.6 ± 3.4	32.3 ± 2.8
	Henry Kelsey	7	2	4	42.5 ± 3.8	37.7 ± 3.2
	Honeysweet	19	2	4	40.4 ± 2.4	23.8 ± 2.4
	JACbow (Kaleidoscope[d])	19	2	4	37.7 ± 2.7	38.5 ± 2.6
	John Cabot	7	2	4	40.8 ± 2.3	30.9 ± 2.1
	KORlore (Folklore)	12	2	4	40.8 ± 5.2	27.1 ± 2.6
	L83[e]	7	2	4	39.5 ± 1.9	24.4 ± 2.7
	Lakeshore Louise	19	2	4	40.0 ± 1.6	26.0 ± 2.0
	MACauck (Olympiad[d])	12	2	4	42.3 ± 2.3	35.5 ± 3.1
	MEIpitac (Carefree Wonder[d])	19	2	4	40.4 ± 3.5	35.0 ± 1.6
	MEIpoque (Pink Meidiland)	19	2	4	37.9 ± 4.4	35.8 ± 4.2
	Morden Blush	19	2	4	40.3 ± 2.3	31.8 ± 2.3
		$2n$ pollen	4		49.0 ± 2.7	
	Morden Centennial	19	2	4	40.3 ± 3.2	36.0 ± 2.3
	Morden Ruby	19	2	4	39.9 ± 3.0	37.0 ± 2.8
	Morden Sunrise	19	2	4	39.3 ± 3.0	35.6 ± 4.5
	MORdust (Star Dust)	4	2	4	41.5 ± 3.1	34.0 ± 3.2
	MORgoldart (Splish Splash)	15	2	4	42.8 ± 3.8	34.3 ± 3.2
	MORthirthree (Persian Autumn)	19	2	4	40.7 ± 2.4	36.4 ± 2.8
	NOAre (Flower Carpet Red)	19	2	4	41.2 ± 3.0	36.8 ± 5.1
	Orange Honey	15	2	4	40.5 ± 3.2	29.8 ± 1.5
	Paloma Blanca	19	3	6	44.4 ± 2.7	25.4 ± 1.9
	Prairie Princess	19	2	4	39.0 ± 3.3	29.2 ± 2.1
	Prairie Wren	19	2	4	38.3 ± 2.8	29.2 ± 2.8
	R. arkansana Porter-1	20	2	4	36.3 ± 1.7	25.5 ± 2.0
	R. arkansana-2	20	2	4	36.4 ± 1.9	26.8 ± 2.4
	R. macrantha Desportes	20	3	6	44.3 ± 3.0	27.6 ± 2.2
	R. palustris Marsh.-1	20	1	2	35.4 ± 1.6	29.2 ± 2.0
	R. palustris-2	20	1	2	35.5 ± 1.8	25.4 ± 2.4
	RADramblin (Ramblin Red)	14	2	4	40.8 ± 2.8	30.9 ± 3.5
	RADsun (Carefree Sunshine)	19	2	4	42.7 ± 2.4	27.4 ± 2.2
	Rise 'n' Shine	15	3	6	43.7 ± 3.8	32.8 ± 1.9
	Royal Edward	19	2	4	40.4 ± 3.4	39.0 ± 4.1
	Royal Occasion	2	2	4	38.1 ± 2.3	29.1 ± 2.0
	SAValife (Rainbow's End)	15	2	4	42.0 ± 3.3	41.6 ± 3.5
	SCRivluv (Baby Love)	15	2	4	40.1 ± 3.2	35.0 ± 4.1
	Shoreside Sam	19	2	4	36.9 ± 1.5	27.1 ± 2.1
	Summer Wind	19	2	4	39.4 ± 2.2	24.2 ± 2.2
	Suzanne	11	2	4	38.8 ± 2.9	26.6 ± 2.0
	TWOadvance (All that Jazz[d])	19	2	4	38.1 ± 2.8	37.9 ± 2.8
	Virginia Reel	19	2	4	39.2 ± 3.9	37.6 ± 3.0
	WEKsacsoul (Bee Bop)	19	2	4	41.7 ± 2.3	39.1 ± 2.3
	Wildenfels Gelb	5	2	4	39.5 ± 3.4	29.0 ± 2.0
	William Baffin	7	2	4	41.0 ± 3.6	33.1 ± 2.0
	William Booth	19	2	4	39.9 ± 2.4	34.5 ± 2.0
	Winnipeg Parks	19	2	4	39.6 ± 3.0	33.8 ± 3.2
	ZLEhoney (Honeybee)	16	2	4	36.8 ± 3.1	25.8 ± 2.2
Pentaploid	Andersonii	19	2		38.9 ± 3.7	28.9 ± 2.6
	R. rubiginosa L.-1	20	4		50.6 ± 3.0	31.1 ± 2.6
	R. rubiginosa-2	20	1		35.0 ± 2.0	29.2 ± 2.2
Hexaploid	Alba Semi-plena	1	2	6	41.6 ± 4.1	33.9 ± 3.8
	Maiden's Blush	1	2	6	42.8 ± 2.6	33.9 ± 1.8
	R. acicularis Lindl.	20	3	6	45.6 ± 2.2	28.0 ± 2.6
	R. woodsii Lindl.-2	20	3	6	43.7 ± 2.7	22.4 ± 1.7
		$4n$ pollen	12		72.7 ± 6.1	
Octoploid	Kinistino, *R. acicularis* selection	20	4	8	48.4 ± 1.8	31.8 ± 3.0

[a] Cultivar name is followed by trademark or exhibition name, if different, in parenthesis.
[b] 1 Alba; 2 Floribunda of climbing floribunda; 3 Grandiflora; 4 Hybrid bracteata; 5 Hybrid foetida; 6 Hybrid gallica; 7 Hybrid kordesii; 8 Hybrid musk; 9 Hybrid nitida; 10 Hybrid rugosa; 11 Hybrid spinosissima; 12 Hybrid tea; 13 Hybrid wichurana; 14 Large flowered climber; 15 Miniature or climbing miniature; 16 Miniflora; 17 Moss; 18 Polyantha; 19 Shrub; 20 Species.
[c] Numbers (i.e. -1 and -2) following species indicate different genotypes.
[d] Winner of the All-America Rose Selection award.
[e] Germplasm release; Svejda 1988.

having proposed sporophytic ploidy levels), the mean pollen diameter ranges between diploid/tetraploid and tetraploid/hexaploid roses overlapped (**Fig. 1**). Overlap in pollen diameter between diploid and tetraploid roses (barring *Caninae* section roses) occurs between 35.2-35.9 µm with five diploid and five tetraploid roses within this range. Overlap between tetraploid and hexaploid roses occurred between 43.7-46.0 µm with 11 tetraploids within this range (out of 202 tetraploids) and five hexaploids (out of six hexaploids).

The presence of some distinctly larger, $2n$ pollen was found among diploid (n=6), tetraploid (n=5), and hexaploid (n=1) roses (**Tables 2-4**). Two roses (2L24 and *R. woodsii*-2) produced some much larger pollen near the size expected

Table 4 Pollen diameter, guard cell length, ploidy predictions, and pedigrees for rose breeding lines grouped by confirmed sporophytic ploidy.

Breeding line	Predicted ploidy (x)		Mean ± SD (µm)		Pedigree[a]
	Gametophyte	Sporophyte	Pollen	Guard cell	
Diploid					
1A114	1	2	33.7 ± 2.2	20.6 ± 1.3	95-1 x Verden
1H148	1	2	34.3 ± 2.0	22.1 ± 1.9	Robin Hood x *Rosa chinensis minima*
1J26	1	2	32.0 ± 2.4	17.1 ± 2.0	*R. chinensis minima* x Thérèse Bugnet
1-2-1J26	1	2	33.7 ± 2.1	23.4 ± 1.5	(1J26 op[b]) op
2-2-1J26	1	2	31.4 ± 1.7	28.6 ± 3.1	(1J26 op) op
2J26	1	2	32.9 ± 1.7	19.2 ± 1.3	*R. chinensis minima* x Robin Hood
1M22	1	2	32.1 ± 2.7	20.4 ± 1.5	Lillian Gibson x *R. chinensis minima*
1N53	1	2	32.3 ± 1.6	30.9 ± 3.0	Max Graf x *R. chinensis minima*
2*n* pollen	2		37.6 ± 1.5		
1V41	1	2	32.1 ± 1.9	27.7 ± 3.2	(Yvonne Rabier op) op
2V41	1	2	33.1 ± 2.2	27.7 ± 2.3	(Yvonne Rabier op) op
1W13	1	2	32.1 ± 1.7	26.8 ± 2.1	1G84 op
2*n* pollen	2		38.3 ± 1.8		
1990-4	1	2	32.0 ± 1.5	21.4 ± 2.3	I.T.-9 x I.T.-18[c]
1995-1	1	2	32.7 ± 1.6	19.0 ± 1.8	*R. rugosa rubra* op
1995-3	1	2	34.6 ± 1.4	22.6 ± 2.3	*R. chinensis minima* selection
1995-4	1	2	33.3 ± 1.5	24.4 ± 1.3	*R. chinensis minima* selection
1998-1	1	2	32.1 ± 1.8	25.8 ± 1.6	(*R. rugosa rubra* x *R. blanda*) op
1998-2	1	2	32.8 ± 2.0	20.3 ± 1.6	*R. rugosa rubra* x *R. chinensis minima*
2*n* pollen	2		38.7 ± 1.4		
1998-4	1	2	30.2 ± 2.4	17.7 ± 1.8	*R. rugosa rubra* x *R. chinensis minima*
2-2000-0067-11	1	2	28.5 ± 2.9	20.0 ± 2.3	(*R. setigera* x 95-1) op
1-2-2000-0067-11	1	2	30.7 ± 2.6	24.5 ± 2.6	2-2000-0067-11 op
2003-1	1	2	30.3 ± 1.8	26.3 ± 2.2	*R. chinensis minima x* Thérèse Bugnet
2005-61	1	2	32.3 ± 1.6	25.4 ± 2.4	((*R. setigera* x 95-1) op) x PolyA
2-set mon	1	2	29.0 ± 1.6	17.0 ± 2.7	(*R. setigera* x 95-1) op
3BA1	1	2	29.4 ± 1.8	21.9 ± 2.5	95-1 op
107-02-01	1	2	30.8 ± 3.1	26.5 ± 2.8	(Etoile Luisante x Sierra Snowstorm) x MORyears
Cantaop1	1	2	34.1 ± 1.9	21.7 ± 1.3	Cantabrigiensis op
H93	1	2	31.2 ± 2.6	22.4 ± 1.6	Haploid of Dorcas
Hugop2	1	2	34.3 ± 1.5	23.9 ± 1.4	*R. hugonis* op
Jrug-2005	1	2	32.5 ± 1.8	19.9 ± 2.8	((Will Alderman op) op)
PolyA	1	2	31.4 ± 1.7	18.6 ± 2.0	(95-1 op) op
1PolyA	1	2	32.1 ± 1.6	27.1 ± 3.8	PolyA op
1PolyF	1	2	32.9 ± 3.2	24.2 ± 2.6	((95-1 op) op) op
Rosa 123	1	2	32.3 ± 2.0	30.6 ± 2.9	Nastarana x (The Fairy x unknown polyantha)
Rosa 215	1	2	30.4 ± 1.5	29.5 ± 2.1	Unknown polyantha x Mevrouw Nathalie Nypels
Rosa 251	1	2	30.4 ± 1.7	23.6 ± 3.0	Unknown polyantha x Mevrouw Nathalie Nypels
Rosa 295	1	2	31.2 ± 1.3	28.8 ± 3.4	La Marne x unknown polyantha
Rosa 320	1	2	31.0 ± 1.7	30.6 ± 2.7	La Marne x unknown polyantha
NW-1	1	2	33.3 ± 2.1	34.0 ± 2.9	Nearly Wild op
2*n* pollen	3		43.7 ± 3.1		
Polybuck1x	1	2	33.6 ± 1.4	25.0 ± 1.5	*R. chinensis minima* x 1990-4
Ser-7	1	2	33.0 ± 1.9	22.8 ± 2.3	*R. sericea ptericantha* op
Triploid					
1B43	2	4	41.1 ± 2.9	21.5 ± 1.5	Rise 'n' Shine x Yvonne Rabier
1G84	2	4	39.1 ± 3.3	27.8 ± 3.3	Orange Honey x 4BA3[d]
2G102	2	4	40.2 ± 2.5	32.1 ± 4.1	Rise 'n' Shine x bulked pollen source
1G177	2	4	39.7 ± 7.1	35.2 ± 2.0	MORgoldart x *R. chinensis minima*
1G181	2	4	42.1 ± 5.4	38.4 ± 2.4	MORgoldart x *R. chinensis minima*
1-1J26	1	2	35.0 ± 4.4	23.8 ± 1.5	1J26 op
1L41-H1	2	4	38.0 ± 5.3	31.8 ± 3.1	1990-1 x Lakeshore Louise
1P27	2	4	40.6 ± 2.4	29.1 ± 3.6	1A28 x 1A10
1R42	3	6	44.9 ± 5.4	35.0 ± 1.2	1990-1 x (MORgoldart x William Booth)
1T26	2	4	40.5 ± 2.7	35.1 ± 3.3	1B30 x 1990-6
1T52	2	4	43.1 ± 2.8	41.6 ± 3.3	(CURlem x George Vancouver) x 1B30
1T70	2	4	38.3 ± 1.7	33.2 ± 2.4	1G148 x 1G59
1V8	2	4	37.7 ± 3.9	33.0 ± 2.3	2G102 op
1X27	2	4	41.6 ± 2.3	41.0 ± 3.0	1B30 x 7A89
2X80	2	4	40.6 ± 4.4	36.0 ± 2.6	BUCbi x KORbin
3X81	2	4	41.8 ± 4.4	35.5 ± 2.9	BUCbi x 1G84
6X81	3	6	44.5 ± 4.6	38.7 ± 3.7	BUCbi x 1G84
4X82	2	4	36.9 ± 2.5	35.6 ± 3.4	BUCbi x 2G102
7X82	2	4	43.1 ± 4.8	43.1 ± 3.7	BUCbi x 2G102
9X82	2	4	38.2 ± 3.1	32.2 ± 2.8	BUCbi x 2G102
1989-1	2	4	42.2 ± 6.0	28.9 ± 2.3	*R. chinensis minima* op
1990-6	3	6	44.0 ± 2.6	31.9 ± 2.3	MEIdomonac x TANnacht
1999-1	2	4	41.2 ± 2.3	36.3 ± 4.2	*R. chinensis minima* x Champlain
HugHaid	1	2	35.4 ± 2.4	26.6 ± 1.8	*R. hugonis* x Haidee
Jdopred	2	4	39.4 ± 2.4	30.1 ± 2.0	John Davis op

Table 4 (Cont.)

Breeding line	Predicted ploidy (x)		Mean ± SD (µm)		Pedigree[a]
	Gametophyte	Sporophyte	Pollen	Guard cell	
NW-2	1	2	34.2 ± 3.1	28.8 ± 2.1	Nearly Wild op
PolyMartyd	1	2	35.4 ± 4.0	28.4 ± 3.4	*R. chinensis minima* x bulked 4x parents
Rhsw	1	2	35.7 ± 2.2	25.1 ± 2.0	Robin Hood x Summerwind
Rosa 313	2	4	37.0 ± 3.8	33.3 ± 1.7	The Fairy x POUlino
Rosa 340	2	4	37.1 ± 1.9	33.2 ± 2.8	(Country Dancer x (*R. palustris*-1 x John Cabot)) x (Spanish Rhapsody x (Applejack op))
Rosa 341	2	4	41.2 ± 3.6	34.6 ± 3.6	SCRivluv x (Folksinger x John Davis)
Rosa 343	2	4	39.1 ± 3.9	33.2 ± 2.2	SCRivluv x Morden Sunrise
Tetraploid					
1A5	2	4	37.8 ± 2.4	28.4 ± 1.4	BUCbi x *R. virginiana*
1A10	2	4	39.6 ± 2.3	30.2 ± 1.8	BUCbi x (Prairie Princess x (*R. palustris*-1 x John Cabot))
4A10	2	4	38.7 ± 2.2	22.5 ± 1.9	BUCbi x (Prairie Princess x (*R. palustris*-1 x John Cabot))
1A28	2	4	40.4 ± 2.6	40.2 ± 4.1	(BUCbi x Summer Snow) x (Chorale x William Baffin)
4A29	2	4	41.6 ± 3.3	25.0 ± 1.2	(BUCbi x Summer Snow) x (Prairie Princess x (*R. palustris*-1 x John Cabot))
1A80	2	4	37.4 ± 1.5	36.6 ± 3.4	George Vancouver x Alba Semi-plena
1A83	2	4	40.2 ± 2.3	32.7 ± 2.2	George Vancouver x *R. virginiana*-2
7A89	2	4	42.3 ± 2.7	34.3 ± 2.1	Hawkeye Belle x William Booth
1B22	2	4	38.7 ± 2.6	43.5 ± 4.4	Orange Honey x (BUCbi x William Baffin)
1B30	2	4	39.2 ± 1.9	27.3 ± 2.3	Orange Honey x (Spanish Rhapsody x (Applejack op))
1B35	2	4	41.1 ± 2.9	33.7 ± 4.1	Rise 'n' Shine x SCRivluv
1B38	2	4	39.9 ± 2.3	38.0 ± 3.6	Rise 'n' Shine x George Vancouver
2n pollen	4		52.4 ± 2.6		
1G18	3	6	44.6 ± 2.8	34.4 ± 4.9	CURlem x 1999-1
2G18	2	4	41.4 ± 2.9	30.5 ± 2.5	CURlem x 1999-1
1G24	2	4	40.9 ± 2.3	23.6 ± 1.6	CURlem x 1999-2
1G59	2	4	40.1 ± 1.7	28.0 ± 1.2	Orange Honey x 1998-3
2G66	2	4	41.5 ± 2.1	27.7 ± 3.6	Orange Honey x 1999-1
6G66	2	4	42.4 ± 2.4	32.2 ± 2.3	Orange Honey x 1999-1
1G109	2	4	42.1 ± 2.2	33.5 ± 2.4	Rise 'n' Shine x (JACient x (*R. laxa* x *R. rubrifolia*))
1G148	2	4	37.9 ± 2.7	37.7 ± 3.9	MORdora x bulk pollen source
6H109	2	4	38.4 ± 4.2	33.7 ± 3.8	George Vancouver x Frontenac
3H130	2	4	42.3 ± 2.6	32.9 ± 2.7	George Vancouver x (Chorale x Suzanne)
3H149	2	4	42.9 ± 2.1	31.9 ± 2.5	Robin Hood x bulked 4x parents
1I4	1	2	34.6 ± 3.6	22.2 ± 1.6	*R. pomifera* x *R. chinensis minima*
3K20	3	6	44.1 ± 1.9	25.6 ± 1.9	Max Graf x bulked 4x parents
1L4	3	6	43.7 ± 3.4	32.0 ± 2.6	((BUCbi X Summer Snow) x (unknown x (*R. palustris-1* x John Cabot)) x RADsun
1L24	2	4	39.3 ± 2.1	29.7 ± 3.4	BUCbi x (Hawkeye Belle x William Booth)
2L24	2	4	38.4 ± 6.2	34.2 ± 2.9	BUCbi x (Hawkeye Belle x William Booth)
4n pollen	8		56.7 ± 4.4		
1L38-H1	2	4	40.1 ± 2.9	29.6 ± 3.0	1990-1 x RADsun
2L38-H2	2	4	41.5 ± 3.2	27.3 ± 1.7	1990-1 x RADsun
10N22	2	4	43.0 ± 3.8	35.1 ± 2.8	1B30 op
1N36	2	4	40.7 ± 4.5	25.1 ± 1.6	(MORgoldart x 1999-1) op
1N39	2	4	40.1 ± 3.2	30.5 ± 2.0	MORgoldart x *R. chinensis minima* (4x)
10P24	2	4	40.1 ± 2.0	32.0 ± 2.4	((BUCbi x Summer Snow) x (Chorale x *R. virginiana*)) x 1A83
1P30	2	4	40.2 ± 2.4	21.6 ± 2.8	Full sibling of 1A28 x (Hawkeye Belle x William Booth)
4P30	2	4	39.5 ± 2.2	23.8 ± 0.8	Full sibling of 1A28 x (Hawkeye Belle x William Booth)
1P38	2	4	42.1 ± 2.4	27.7 ± 1.5	((BUCbi x Summer Snow) x (unknown x (*R. palustris-1* x John Cabot))) x 1998-3
1P72	2	4	42.2 ± 3.0	25.8 ± 1.7	(1990-1 x William Booth) x (Dorcas x (Rise 'N Shine x SCRivluv))
2P118	2	4	38.8 ± 2.1	26.3 ± 3.0	(1994-1 x (Chorale x *R. virginiana*-1)) x (Hawkeye Belle x (full sibling of 1990-1 x MORcarlet))
10Q2	2	4	41.6 ± 2.0	23.8 ± 1.2	1B22 x 1998-3
11Q2	2	4	39.5 ± 2.1	25.3 ± 1.6	1B22 x 1998-3
2Q12	2	4	37.8 ± 2.4	38.4 ± 3.2	1B30 x (MORdora x 1999-1)
10Q12	2	4	42.1 ± 3.1	26.5 ± 2.5	1B30 x (MORdora x 1999-1)
11Q12	2	4	41.2 ± 2.1	28.1 ± 1.7	1B30 x (MORdora x 1999-1)
1Q16	2	4	40.0 ± 3.5	28.5 ± 2.0	1B30 x (full sibling of 1990-1 x MORcarlet)
1Q18	2	4	41.4 ± 2.1	27.4 ± 1.7	1B30 x RADsun
1Q30	2	4	40.6 ± 2.5	43.5 ± 2.8	1B38 x (George Vancouver x William Booth)
10Q30	2	4	38.3 ± 2.3	26.5 ± 2.5	1B38 x (George Vancouver x William Booth)

Table 4 (Cont.)

Breeding line	**Predicted ploidy (*x*)**		**Mean ± SD (µm)**		**Pedigree**[a]
	Gametophyte	**Sporophyte**	**Pollen**	**Guard cell**	
1Q33	2	4	41.8 ± 2.8	26.2 ± 3.4	1B38 x 1G102
1Q63	2	4	40.7 ± 2.0	27.5 ± 1.7	(MORgoldart x 1999-1) x 1998-3
1R32	2	4	42.5 ± 2.3	27.3 ± 3.3	1998-3 x 1999-1
1R43	2	4	41.5 ± 3.2	32.6 ± 0.9	1990-1 x 2G18
2R65	2	4	37.5 ± 1.9	26.3 ± 1.9	*R. arkansana*-1 x 1A83
1S23	2	4	40.0 ± 2.9	35.0 ± 2.6	1A10 x 1B30
1S62	2	4	39.8 ± 5.6	41.0 ± 4.0	((BUCbi x Summer Snow) x William Booth) x (CURlem x *R. chinensis minima*)
2T13	2	4	38.8 ± 3.8	35.2 ± 2.9	1B30 x (CURlem x *R. chinensis minima*)
1T20	2	4	40.9 ± 2.5	37.1 ± 3.5	1B30 x 1L24
1T30	2	4	40.2 ± 3.6	42.0 ± 4.4	1B30 x Dorcas
2T34	2	4	42.4 ± 4.2	42.3 ± 5.1	1B30 x 1990-2
3T34	2	4	41.9 ± 3.0	42.8 ± 2.1	1B30 x 1990-2
5T34	2	4	41.8 ± 3.5	34.1 ± 2.8	1B30 x 1990-2
1T38PIC	2	4	40.1 ± 2.6	40.0 ± 2.6	1B30 x Paloma Blanca
2T38	2	4	40.7 ± 3.2	37.0 ± 2.7	1B30 x Paloma Blanca
2T52	2	4	38.5 ± 2.2	29.9 ± 3.0	(CURlem x George Vancouver) x 1B30
2T93	2	4	43.2 ± 2.4	31.7 ± 4.2	(George Vancouver x (Chorale x Suzanne)) x AUSclough
1U4	2	4	40.3 ± 3.5	38.6 ± 3.7	BUCbi x (George Vancouver x Peace)
1U10	2	4	37.9 ± 3.9	23.8 ± 1.5	BUCbi x *Rubus odoratus*[e]
2U10	2	4	37.8 ± 3.2	24.4 ± 1.4	BUCbi x *Rubus odoratus*
1U23	2	4	38.1 ± 2.0	28.9 ± 2.0	1990-1 x 1B30
2U23	2	4	39.3 ± 2.7	37.9 ± 3.7	1990-1 x 1B30
1U34	2	4	42.5 ± 2.3	31.2 ± 4.5	1990-1 x (George Vancouver x Frontenac)
1U55	3	6	46.0 ± 3.9	29.1 ± 2.7	Rise 'n' Shine x *R. primula*
1V12	3	6	45.6 ± 3.3	35.1 ± 2.6	1H109 op
5X81	1	2	35.7 ± 2.9	34.3 ± 2.6	BUCbi x 1G84
2X82	2	4	40.9 ± 7.4	39.1 ± 3.4	BUCbi x 2G102
1990-1	2	4	41.1 ± 2.1	29.9 ± 1.7	Goldilocks x (Proud Land x Pizzicato)
1990-2	2	4	43.1 ± 3.4	27.0 ± 2.6	JACdew x (unknown shrub rose x Don Juan)
1990-3	2	4	40.1 ± 2.7	27.3 ± 2.1	MACwaihe x (unknown shrub rose x Don Juan)
1990-5	2	4	37.0 ± 1.8	23.0 ± 1.0	BUCbi x (Little Darling x (unknown shrub rose x Don Juan)
1992-1	2	4	40.6 ± 2.2	29.0 ± 3.3	Little Darling x William Baffin
1994-1	2	4	40.1 ± 2.4	25.5 ± 2.1	BUCbi x Hawkeye Belle
1998-3	2	4	38.8 ± 2.4	30.9 ± 1.5	(BUCbi x William Baffin) x Crimson Glory
1999-2	2	4	38.4 ± 2.3	21.2 ± 1.9	*R. virginiana*-2 x *R. laxa*
1999-3	2	4	40.8 ± 2.7	35.2 ± 2.6	Champlain x William Baffin
2000-1	2	4	37.6 ± 2.3	24.3 ± 1.8	Basye's thornless op
2000-2	2	4	37.0 ± 2.2	25.9 ± 1.9	(Flora McIvor op) op
2001-0830-trif	2	4	40.9 ± 2.9	28.3 ± 3.0	Induced polyploid of (Natchez x (The Fairy x unknown polyantha))
Jdopgreen	2	4	38.2 ± 2.5	27.7 ± 2.5	John Davis op
Lockh-Liza	2	4	40.6 ± 3.7	38.2 ± 3.0	KORruge x MOEwinst
Moenut	2	4	39.1 ± 2.5	28.0 ± 2.3	*R. nutkana* op
Moore-1	2	4	39.6 ± 2.7	37.8 ± 2.3	(Little Darling x Yellow Magic) x (Anytime x Tigris)
Lockh-egob	1	2	35.3 ± 2.9	37.7 ± 2.9	unknown
Stell-2	2	4	38.5 ± 2.7	42.6 ± 2.7	*R. stellata mirifica* op
Pentaploid					
1C5	4	unknown	47.0 ± 4.4	28.1 ± 2.8	*R. rubiginosa* x *R. pomifera*
Nut3	2	4	41.2 ± 3.1	17.5 ± 1.8	*R. nutkana* op
Hexaploid					
1I2	3	unknown	44.3 ± 3.3	37.4 ± 3.0	*R. rubiginosa* x (Spanish Rhapsody x (Applejack op))
1988-1	2	unknown	41.5 ± 4.7	30.4 ± 3.4	*R. rubiginosa* x Haidee
2002-1	4	8	48.8 ± 2.7	42.9 ± 3.2	(*R. setigera* x 95-1) op

[a] Variety names (not trademark names) are provided and can be cross referenced in Cairns (2000) and advance selection designations can be cross referenced within this table.
[b] op, open-pollinated.
[c] Buck 1978.
[d] Zlesak *et al*. 2005.
[e] Intergeneric crosses were attempted with the goal of generating diploids. Morphological features are that of only rose.

for $4n$ pollen and were classified as such (**Tables 3** and **4**). In addition, the tetraploid cultivar Frontenac had extremely large pollen (73.1 µM), larger than expected for $4n$ ($8x$) pollen (~56.7 µM; **Table 6**). Since the gametophytic ploidy (x) and possible origin (n) of the large pollen of 'Frontenac' remains unclear, the ploidy of the large pollen was not estimated and therefore not used in analyses. In addition, a clear distinction in size between n and $2n$ pollen, as found in diploid and tetraploid roses, was not generally found in triploids where there tended to be a wide distribution within and between triploid genotypes for pollen diameter (**Tables 2-4; Figs. 2** and **3**). Some triploid roses may also be producing $2n$ pollen, but with the variability in pollen size it was difficult to confidently identify and distinguish $2n$ pollen from possibly aneuploid pollen.

The mean diameter of $1x$ pollen was 32.6µm (pollen from diploids and typical *Caninae* section species), and this value was used to calculate the predicted diameters of 2, 3,

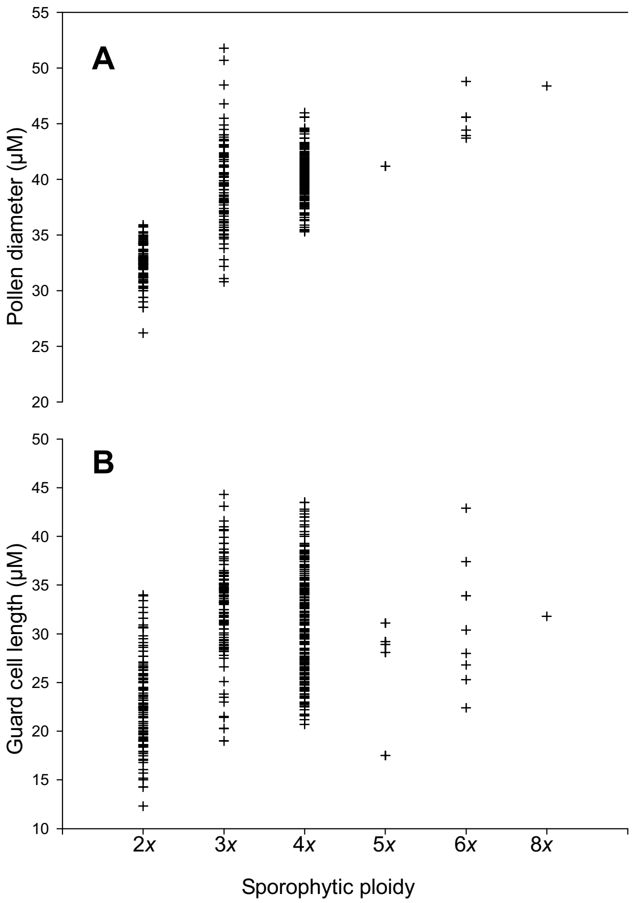

Fig. 2 Mean pollen diameter (A) (*Caninae* section species and hybrids removed from pollen diameter data due to unbalanced meiosis) and mean guard cell length (B) for roses grouped by sporophytic ploidy.

4, 5, 6, 8, and 12*x* pollen (**Table 6**). Triploid and pentaploid genotypes (not from the *Caninae* section), *Caninae* section rose species or hybrids with unexpectedly large pollen (*R. rubiginosa*-1, 1C5, 1I2, 1988-1), and the pollen of 'Frontenac' (unexpectedly large without being near the sizes expected for 2*n* or 4*n* pollen) were omitted due to ambiguity of the gametophytic ploidy level of stainable pollen. Observed pollen diameter was consistently less than predicted diameter and observed diameter ranged from 87.0-97.8% of predicted diameter (**Table 6**). The Pearson's correlation coefficient between actual and predicted mean pollen diameter across gametophytic ploidy levels was r=0.98 (P<0.001), indicating a very strong linear association between pollen volume and gametophytic ploidy (**Table 6**).

Species roses were grouped according to section to explore trends by section for pollen diameter and guard cell length (**Table 7**). ANOVA revealed that section (F=2.1; P= 0.17) and species nested in section (F=2.8; P=0.06) were not significant factors. For guard cell length section (F=0.7; P=0.68) and species (F=1.9; P=0.17) were also not significant. Section *Banksiana* had smaller pollen than all the other sections, but with only a single genotype represented, it was omitted from the analysis.

Ploidy level varied among individuals within horticultural rose classes (**Table 8**). Multiple ploidy levels were represented within each of the five largest classes based on greatest number of registered cultivars (floribunda, grandiflora, hybrid tea, miniature, and shrub; Cairns 2000). Triploid cultivars were especially prevalent among shrub roses sold for use as low-maintenance landscape plants. For instance, most or all of the shrub roses assessed within the Knock Out® series, Flower Carpet® series, and from the House of Meilland (variety names begin with MEI) are triploid (**Table 3**). Additionally, of the eleven shrub roses which have won the prestigious All-America Rose Selections award, eight of them are triploid (**Table 3**).

Ploidy transmission from triploids

In crosses between tetraploid female and triploid male parents, both tetraploid (n=20) and triploid (n=23) offspring were found in an approximately equal proportion (**Table 9**). A scatterplot displaying pollen diameter of the 30 pollen grains assessed for each triploid parent is presented in **Fig. 3**. Pollen grains were observed the size expected for 1*x*, 2*x*, and ≥3*x* pollen among the 30 pollen grains sampled for triploids used in pollinations. Triploid males 1G84, 2G102, and 1990-6 crossed onto tetraploid females produced both tetraploid and triploid offspring. 'KORbin' and 1B43 produced only triploid or tetraploid offspring, respectively, however, progeny sizes were small (n=2 per male).

Triploid parents served as a bridge between ploidy levels. For instance, among limited progeny numbers of open-pollinated seedlings of triploid females, diploid (1W13 and NW-1), triploid (1V8, Jdopred, and NW-2), and tetraploid (Jdopgreen) progeny were recovered (**Table 4**). Triploid progeny were also obtained from multiple parental ploidy combinations (**Table 4**). Triploids were generated, as expected, from crosses between diploid and tetraploid

Table 5 Frequency of roses[a] within each predicted versus actual sporophytic ploidy level based on pollen diameter along with percentage accuracy.

Actual ploidy	Predicted ploidy				Total	% Accuracy
	2*x*	4*x*	6*x*	8*x*		
2*x*	69	0	0	0	69	100.0
3*x*	16	69	9	4	98	---
4*x*	5	164	11	0	180	91.1
5*x*	0	1	0	0	1	---
6*x*	0	0	4	1	5	80.0
8*x*	0	0	0	1	1	100.0
Total	90	234	24	6	354	

[a] *Caninae* section species and their hybrids (other than Alba roses) were omitted due to unusual meiosis making sporophytic ploidy prediction from pollen diameter uncertain.

Table 6 Rose pollen ploidy and its relationship to pollen volume.

Pollen ploidy[a]	1*x*	2*x*	3*x*	4*x*	8*x*	12*x*
No. of samples	107.0	211.0	5.0	6.0	1.0	1.0
Observed diameter	32.6	40.2	45.3	49.6	56.7	72.7
S.D.	1.7	2.0	2.1	1.6		
Predicted diameter[b]	---	41.1	47.0	51.7	65.2	74.6
Difference		0.9	1.7	2.1	8.5	1.9
Observed % of predicted		97.8	96.4	95.9	87.0	97.5

[a] Pollen ploidy includes *n* and suspected 2*n* and 4*n* pollen. Pollen from triploids and pentaploids, polyploid hybrids derived in part from *Caninae* section species (other than Albas), and the large pollen of 'Frontenac' were omitted because of uncertain pollen ploidy classification.
[b] Predicted diameter is calculated by multiplying the actual diameter of 1*x* pollen by the cubed root of pollen ploidy and is based on the assumption that pollen volume is proportional to ploidy.

Table 7 Standardized pollen diameter and actual guard cell length for rose species grouped by subgenus and section.

Subgenus	Section	No. species	No. genotypes	Mean[a] ± SD (µm)	
				Pollen[b]	Guard cell
Rosa	*Banksianae*	1	1	26.2	19.4
	Caninae	4	7	34.4 ± 1.4 b	24.7 ± 3.0
	Gallicanae	1	1	33.0 b	24.7
	Pimpinellifoliae	4	5	33.2 ± 1.8	24.5 ± 4.4
	Rosa[c]	15	22	31.7 ± 2.0	23.8 ± 4.3
	Synstylae	3	3	34.2 ± 0.9	23.2 ± 4.2
Platyrhodon	*Microphyllae*	1	1	32.6	21.4

[a] Genotype is the experimental unit.
[b] Pollen diameter was standardized to represent what is expected for 1*x* pollen.
[c] Syn. *Cinnamomeae*.

Table 8 Frequency of sporophytic ploidy level for rose cultivars and species genotypes grouped by horticultural class.

Horticultural class	Sporophytic ploidy level						Total
	2*x*	3*x*	4*x*	5*x*	6*x*	8*x*	
Species roses							
Species	17	2	18	2	4	1	44
Old garden roses (class in existence before 1867)							
Alba					2		2
Damask			2				2
Hybrid bracteata		1	1				2
Hybrid foetida			2				2
Hybrid gallica			2				2
Hybrid perpetual			1				1
Hybrid spinosissima		1	4				5
Miscellaneous old garden rose			1				1
Moss			2				2
Noisette	1						1
Modern roses (class in existence as of 1867)							
Floribunda	1	5	3				9
Grandiflora		1	2				3
Hybrid kordesii		1	4				5
Hybrid moyesii			2				2
Hybrid musk	2	1					3
Hybrid nitida	1						1
Hybrid rugosa	13	3	1				17
Hybrid tea		2	4				6
Hybrid wichurana		1					1
Large flowered climber	1	3	2				6
Miniature	3	6	7				16
Miniflora			1				1
Polyantha	10	1					11
Shrub	12	44	56	1			113
Total	61	72	115	4	6	1	259

Table 9 Frequency of tetraploid and triploid rose offspring from crosses between tetraploid females and triploid males.

Female (4*x*)	Male (3*x*)	Offspring		Total
		No. 4*x*	No. 3*x*	
BUCbi	KORbin	0	2	2
	1G84	3	5	8
	2G102	5	9	14
	1990-6	0	1	1
1A10	1990-6	1	3	4
4A29	1G84	2	0	2
1B30	1B43	2	0	2
	2G102	1	0	1
	1990-6	6	1	7
1990-1	2G102	0	2	2
Total		20	23	43

parents (1B43, 1G84, 1G177, 1G181, 1999-1, HugHaid, PolyMartyd, and Rhsw). Triploid progeny were also found between crosses of two direct tetraploid parents (1L41-H1, 1P27, 1T52, 1X27, and Rosa 343) or pedigrees that trace back to a tetraploid maternal parent and tetraploid paternal grandparents (1R42). In addition, triploid progeny were found among crosses between tetraploid and triploid parents (1T26, 2X80, 3X81, 6X81, 4X82, 7X82, and 9X82; **Tables 4** and **9**).

Section *Caninae* species, or hybrids involving them, had variable mean pollen diameters suggesting different pollen ploidy across genotypes. All *R. glauca* Pouret, *R. mollis* Smith, and *R. pomifera* genotypes and 1I4 had pollen diameters expected for 1*x* pollen, typical for *Caninae* section species regardless of sporophytic ploidy level (**Tables 2**-**4**). One *R. rubiginosa* genotype (-2) had a mean pollen diameter expected for 1*x* pollen (35.0 µm), while the other (-1) had a mean pollen diameter of 50.6 µm, that expected for 4*x* pollen (**Table 3**). The two alba rose cultivars (Alba Semi-plena and Maiden's Blush; 41.6 µm and 42.8 µm, respectively) and 1988-1 (41.5 µm) had pollen diameters typical for tetraploids (**Tables 3** and **4**). Breeding line 1A80 ('George Vancouver' x 'Alba Semi-plena') is tetraploid (**Table 4**), consistent with 'Alba Semi-plena' producing 2*x* pollen. The observed diameter of 1I2 (44.3 µm) was that expected for 3*x* pollen (**Table 4**).

DISCUSSION

Wide diversity of ploidy within both wild and cultivated roses makes rose a unique crop and useful model from which to study ploidy diversity and ploidy transmission. The diversity of rose cultivars, species, and breeding lines (many having multiple generations of parents characterized

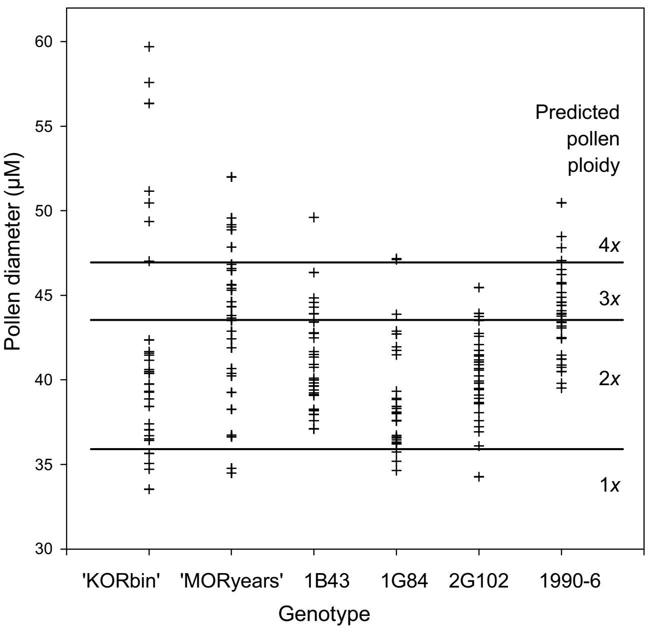

Fig. 3 Scatterplots of pollen diameter (30 grains/genotype) of selected triploid roses with reference to pollen diameter ploidy thresholds used for ploidy prediction.

for ploidy) within this study offers the opportunity for a unique glimpse into ploidy variability and transmission. Direct chromosome counts, although valuable and unparalleled, are time consuming and require individuals with specialized skill. Indirect methods of ploidy assessment can be relatively accurate and in some instances offer added, complementary information that direct chromosome counts may not provide.

Usefulness of indirect ploidy assessments

1. Pollen diameter

Pollen diameter was useful to predict sporophytic ploidy level and gametophytic ploidy level(s) (n, $2n$, and $4n$ pollen) in rose. Pollen diameter data correctly predicted 100% of diploid, 91.1% of tetraploid, 80% of hexaploid, and 100% of octoploid roses expected to have balanced meiosis and not be within or recently derived from section *Caninae* species (**Table 5**). The usefulness of pollen diameter to predict sporophytic ploidy level declines for triploid or pentaploid roses and roses in or recently derived from section *Caninae* species.

Considering data from all roses in this study, except those within or recently derived from *Caninae* species, there was less overlap for pollen diameter between ploidy level ranges between diploid and tetraploid than tetraploid and hexaploid roses (**Fig. 2**). The same trend has been found for pollen diameter ranges between diploid/tetraploid and tetraploid/hexaploid potatoes (Bamberg and Hanneman 1991). These authors concluded that pollen diameter was not effective in separating tetraploids and hexaploids, but was a fast and reliable method to separate diploids from tetraploids or hexaploids. Similar results were observed in this study for rose. The relatively high frequency of triploids in rose versus potato, however, complicates sporophytic ploidy prediction because the pollen diameter range for triploids is variable and crosses all ploidy levels (**Fig. 2**).

Considering pollen diameter data of all roses in this study, except triploids, pentaploids, and those within or recently derived from section *Caninae*, ploidy prediction ranges can better be set at: diploid (<35.6 μm), tetraploid (35.6 μm to <43.7 μm), hexaploid (43.7 to 47.0 μm), and octoploid (>47.0 μm). The modified cutoff (35.6 μm) between diploids and tetraploids is midway between where they overlap (35.2-35.9 μm). Although the other two cutoffs are less clear, 43.7 μm is a useful cutoff between tetraploids and hexaploids because it is the smallest diameter for a hexaploid (*R. woodsii*-2). Between hexaploid and octoploid roses, 47.0 μm is the midpoint between the hexaploid *R. acicularis* (largest hexaploid after 2002-1, a potential outlier, is removed) and octoploid 'Kinistino'. Roses with mean pollen diameters of ≥43.7 μm are recommended for direct chromosome counts because of increased ambiguity in ploidy prediction.

Pollen viability data in conjunction with mean pollen diameter and pollen size distribution within a genotype can be explored as a means to efficiently separate triploid from diploid and tetraploid roses. Pollen diameter alone in roses is not enough to distinguish triploids from other sporophytic ploidy levels. Pollen stainability has been useful for separating triploid from diploid and tetraploid accessions in banana (Tenkouano *et al.* 1998). Triploid roses have been documented as having generally reduced pollen viability and fertility relative to diploid or tetraploid roses; however, pollen viability also varies considerably among rose cultivars regardless of ploidy (Rowley 1960b; Shahare and Shastry 1963; Leus 2005; Crespel *et al.* 2006). Pollen diameter was useful to hypothesize male gametophytic ploidy level of pollen across all sporophytic rose ploidy levels and sections and ultimately may have the greatest practical application to rose breeders.

2. Guard cell length

Guard cell length was not a good general predictor of sporophytic ploidy in rose because of a low Tau's correlation between ploidy and guard cell length (r=0.26; $P<0.001$). For the species assessed in the *R. carolina* complex, their guard cell length did provide useful, predictive ability in separating diploid from tetraploid species as reported by Joly *et al.* (2006). Omitting *R. foliolosa* (2*x*; 26.2 μm), this study also points to a clear distinction in guard cell length range for diploid (16.8-22.8 μm) and tetraploid (24.5-29.2 μm) species within the complex. The unexpected hexaploid, *R. woodsii*-2, had a mean guard cell length (22.4 μm) typical for diploids. The guard cell length of the triploid *R. carolina plena* (28.2 μm) falls within the range for tetraploids. However, the origin of this repeat blooming, double-flowered clone is unclear and the possibility exists that it is a *R. carolina* hybrid (Lynes and Lynes 1955). Although *R. carolina* is reported to be tetraploid by Joly *et al.* (2006) and includes multiple diploid progenitors, the particular clone of *R. carolina* assessed in this study was diploid. *Rosa carolina* is listed as having both diploid and tetraploid forms in Cairns (2000) and highlights the confusion in the literature regarding classification and identification within this complex.

In polyploidization studies using a narrow germplasm base, guard cell length has been useful to separate roses differing in ploidy level in meristematic layer I (Semeniuk and Arisumi 1968; Zlesak *et al.* 2005). Adaptation to environmental conditions, ploidy level, and genetics can all influence stomata size, density, and distribution (Weyers and Meidner 1990). Since roses are native over a wide range of climates and rose cultivars are of complex hybrid origin and trace back to multiple species (Krüssmann 1981), it is understandable that guard cell length could vary greatly across rose germplasm.

3. Flow cytometry

Flow cytometry has been a very useful tool for indirect assessment of ploidy level in rose. However, DNA content can differ widely between individuals at the same ploidy level and complicate ploidy estimation. For instance, Yokoya *et al.* (2000) found that the triploid floribunda 'Frensham' had similar DNA content to the tetraploid species *R. spinosissima* L., and pentaploid *R. canina* had more DNA than the hexaploid *R. moyesii* Hemsley and Wilson. Leus (2005) as well reports complications in ploidy assessment based on DNA content with several roses deviating from the typical DNA content range for given ploidy levels. There are 14 rose cultivars in common between the current study and that of Leus (2005), which solely relies on flow cytometry for ploidy classification. For eleven of the roses in common, direct chromosome counts substantiated ploidy estimates using DNA content as determined by flow cytometry. The three roses which were not in agreement were 'Crimson Shower' (triploid; estimated as diploid by flow cytometry), 'Maiden's Blush' (hexaploid; estimated as tetraploid by flow cytometry), and 'MEIdomonac' (triploid; estimated as tetraploid by DNA content).

Perhaps differences in ploidy assessments between direct chromosome counts and ploidy estimates using flow cytometry are due to ploidy chimeras or different genotypes inadvertently sold under the same cultivar name. Adventitious roots from stem cuttings typically arise from tissue derived from meristematic Layer III (Esau 1977), which is the layer assessed for ploidy using root tip squashes. Flow cytometry, however, can detect ploidy chimeras using macerated leaf tissue derived from all three meristematic layers and these three cultivars were not among the roses classified as ploidy chimeras by Leus (2005).

Within wide or complex interspecific hybrids, as most rose cultivars are, it is common for genomic reorganization to occur and genome size to be altered (Levin 2002). Although flow cytometry is a useful indirect tool for sporophytic ploidy estimation in rose, it can lead to ploidy misclassification, as do ploidy estimates based on pollen diameter, and should be accompanied by direct chromosome counts to confirm ploidy assessment for genotypes where accurate ploidy assessment is imperative. Even so, flow cytometry may have an advantage over pollen diameter to estimate sporophytic ploidy for especially triploid roses, roses within or derived from the section *Caninae*, and ploidy chimeras. This is due to flow cytometry assessing somatic cells typically from leaf tissue derived from all meristematic layers and circumvents complications due to unusual or aberrant meiotic events and observing the resulting gametophytic tissue.

Yokoya *et al*. (2000) found a trend for greater uniformity in DNA content for genotypes within section of *Rosa* rather than between. When considering the relationship between rose section and guard cell length or pollen diameter, significant trends were not found. For instance, the nuclear DNA content determined by flow cytometry of *R. banksiae* (1.04 pg per 2C nucleus; Yokoya *et al*. 2000), the species with the smallest pollen diameter, was similar to or greater than that of other rose species. Besides DNA content, variability for pollen size for plants in general can be attributed to genetic and/or environmental factors (Stanley and Linskins 1974) and may introduce enough variability that there is little to no differentiation in pollen size between germplasm groups. There were no significant differences between rose sections for guard cell length, which, as discussed above, may be due to widespread distribution and environmental adaptation of roses. Wide variability for guard cell length was found across ploidy levels as well (**Tables 2-4; Fig. 2**).

Caninae section species and hybrids

Multiple genomes have been proposed to constitute *Caninae* section species (all are polyploid). There are two copies of the genome which form the bivalent pair and one copy of each of the remaining genomes whose chromosomes are univalents throughout meiosis (Nybom *et al*. 2004). Typically, little morphological variation occurs within a single *Caninae* section species. This has been attributed to the lack of recombination between genomes present in just one copy and the high allelic similarity between the two copies of the genome which preferentially pair during meiosis (Nybom *et al*. 2004). It has been proposed that gene(s) governing *Caninae* meiosis are likely located on the duplicated genome (Wylie 1976; Werlemark 2003). In gynogenetic haploids of *Caninae* section species and intersectional hybrids with *Caninae* section species, changes in dosage of gene(s) governing *Caninae* meiosis may lead to altered expression of *Caninae* meiosis. In addition, dosage changes of these genes governing meiotic pairing in *Caninae* section species are suspected to result in greater environmental sensitivity and greater meiotic variability (Wylie 1975; Werlemark 2003). Most section *Caninae* species genotypes assessed had pollen diameters expected to be 1x; however, one *Caninae* species genotype (*R. rubiginosa*-1; it arrived at the University of Minnesota Landscape Arboretum as open pollinated seed collected from another arboretum, which opens the possibility that it may be an interspecific hybrid) and hybrids involving *Caninae* species ('Andersonii', 1C5, 1I2, and 1988-1) had the expected pollen diameters for 2x, 3x, or 4x pollen (**Tables 2-4**).

Finding relatively uniform pollen the diameter expected for 1x pollen in the intersectional tetraploid hybrid 1I4 (*R. pomifera* x *R. chinensis minima* (Sims) Voss) and the triploid seedling *R. pomifera*-3 suggests that *Caninae* meiosis, which results in 1x pollen, may be functioning in some respect in these genotypes. Perhaps another genome is substituting to form bivalents. *Rosa pomifera*-3 was a twin embryo (*R. pomifera*-2 is its tetraploid twin) in a single achene and may have arisen from a synergid that developed into an embryo without fertilization. *Rosa pomifera*-3 had comparable pollen stainability (>50%) and achenes per hip (~35) to *R. pomifera*-2 (unpublished data). This is unlike the tetraploid *R. canina* plants derived from pentaploid *R. canina* through parthenogenesis by El Mokadem *et al*. (2001) where they found complete pollen abortion, and progeny data when they were used as females suggest only 4x (2n) eggs were functional. The selfed seedlings assessed of *R. pomifera*-3 were triploid, suggesting the possibility of typical *Caninae* meiosis with two genomes forming bivalent pairs during both macro- and microgametogenesis resulting in 2x eggs and 1x pollen. Investigation of which chromosomes and genomes are pairing in these *R. pomifera* genotypes and 1I4 can offer insight into genomic structure, gene(s) governing pairing of homeologous chromosomes, and homology between genomes within section *Caninae* species and between section *Caninae* species genomes and genomes of other species.

Genotype 1988-1 is hexaploid and has pollen the diameter expected for 2x pollen. *Rosa alba* (2n=6x=42) produces 2x pollen (Hurst 1925) and is suspected to be an intersectional cross of a pentaploid *Caninae* section species and *R. gallica* L., a tetraploid species with typical meiosis. In *R. alba* it is suspected that the two *R. gallica* genomes and two of the remaining *Caninae* section genomes preferentially pair during meiosis, resulting in 2x pollen and 4x eggs (Hurst 1925). Breeding line 1988-1 (2n=6x=42) had pollen the diameter expected to be 2x and a similar meiotic situation may be occurring as it is an intersectional cross of section *Caninae* pentaploid *R. rubiginosa* and the tetraploid 'Haidee'. On the other hand, for the hexaploid 1I2 (2n=6x=42), another intersectional *R. rubiginosa* hybrid, the pollen diameter is the size expected for 3x pollen and suggests *Caninae* section meiosis may have been replaced with typical, balanced meiosis. Moreover, *R. rubiginosa*-1 and 1C5 (both 2n=5x=35) have relatively large pollen diameters (50.6 and 47.0 µm, respectively), diameters expected for 4x pollen, the ploidy level of eggs of pentaploid *Caninae* section species. Although both species parents used to produce 1C5 (*R. rubiginosa* and *R. pomifera*) are in the *Caninae* section, large pollen size suggests a deviation from typical *Caninae* meiosis. Nybom *et al*. (2004) suggest that although there is very high similarity between the duplicate genome that preferentially pairs within *Caninae* section species and this duplicated genome is relatively similar across *Caninae* species, there are some species-specific differences within this genome. Genes controlling chromosome pairing in *Caninae* section species may be located on the duplicated, preferentially pairing genome (Wylie 1976), and differences for this genome between *R. rubiginosa* and *R. pomifera* (parents of 1C5) may be great enough to alter typical *Caninae* meiosis. Pollen diameter provides an estimate of gametophytic ploidy and is useful to highlight genotypes which may have aberrant meiosis and warrant further characterization.

Ploidy transmission from triploid parents

Interploidy crosses have been used to bring genetic resources from the diploid level to the tetraploid level in two generations with a triploid intermediary (de Vries and Dubois 1996; Leus 2005). In crosses between tetraploid females and triploid males Leus (2005) found ~98% tetraploid offspring (123/125). Such a high rate of tetraploids differs from what was found in the present study; about half triploid (23/43) and half tetraploid (20/43) offspring (**Table 9**). Leus (2005) reported a lower frequency of tetraploid offspring in triploid x tetraploid crosses (11/15) and approximately half triploid (6/14) and half tetraploid (7/14) from triploid x triploid crosses. Greater pollen vigor has been associated with $2x$ pollen compared to $1x$ pollen in potato (Simon and Peloquin 1976), and greater vigor of $2x$ pollen has been proposed to explain why such a high rate of tetraploid offspring were recovered by Leus (2005) in tetraploid x triploid crosses. Perhaps the difference in the rates of offspring at the tetraploid and triploid levels between Leus (2005) and the current study reflects different pollen application rates and different intensities of pollen competition. Perhaps the current study had a lower pollen application rate and allowed for a closer representation of the viable gametic sample. Greater pollen competition and $2x$ male gametes out-competing $1x$ gametes and more frequently participating in fertilization may also explain why among intermated triploids no diploids were recovered by Leus (2005), while diploids were recovered by Reimann-Philipp (1981). Additionally, the different tetraploid and triploid parents used in these studies could account for differences in ploidy transmission. In this study, population sizes were small for each female, male, and specific cross and limits detection of patterns for ploidy inheritance based on parent(s). Differences among triploid cultivars for pollen diameter distribution and pollen ploidy (including aneuploidy), tetraploid females used, and pollen application rate and pollen competition may all influence how efficiently one can use a triploid to bridge to a particular ploidy level.

Triploids can readily serve as ploidy bridges (Ramsey and Schemske 1998; Leus 2005). Open-pollinated triploid genotypes produced diploid, triploid, and tetraploid seedlings (**Table 4**) (Reimann-Philipp 1981). Triploids crossed with diploids or tetraploids have produced offspring which were diploid, triploid, or tetraploid (**Table 4**) (Barden and Zlesak 2004; Leus 2005). In crosses of the diploid breeding line 0-47-19 (pedigree is *R. wichurana* Crépin x 'Floradora') with the triploid 'MORyears' as a male parent, diploid, triploid, and tetraploid offspring have been recovered, suggesting the possibility of 'MORyears' producing functional $1x$, $2x$, and $3x$ pollen (Barden and Zlesak 2004). Gametes that are $3x$ are possible in triploids through $2n$ gamete formation with mechanisms like parallel spindles leading to balanced, $3x$ gametes more commonly than mechanisms like omission of the second meiotic division (Carputo and Barone 2005). The trend for greater within and between genotype variability for pollen diameter in triploids than in other ploidy levels is in agreement with previous reports (Jacob and Pierret 2000; Leus 2005; Crespel *et al.* 2006) and may reflect pollen grains of different ploidy levels, including aneuploid gametes (**Fig. 2**). Although pollen of variable sizes from triploids are stainable using acetocarmine, many are not functional. Leus (2005) found relatively low *in vitro* pollen germination (0.12-6.93%) from triploids, while diploid and tetraploid controls had much higher *in vitro* germination (14.99-43.11%).

Some triploid parents may be more amenable to producing functional gametes at a particular ploidy level than others. If the goal is diploid offspring from crosses of diploid x triploid parents, triploid parents with relatively small mean pollen diameter like 'Erfurt' (30.8 μm) or 'MEIneble' (32.2 μm) and limited pollen application to minimize pollen competition may prove to be more efficient, while if hexaploid progeny is desired from hexaploid x triploid crosses, roses with relatively larger mean pollen diameter like 'MORtange' (48.5 μm) or 'RADrazz' (46.8 μm) could be more useful. Additionally, sorting and utilizing pollen of desired size with tools such as flow cytometry (Leus 2005) or appropriately sized nylon mesh (Eijlander 1988; Okazaki *et al.* 2005) is also possible. Rowley (1960b) suggests there is a tendency in roses for only the euploid gametes to be functional, which may explain the low pollen viability among triploids. In addition, aneuploidy is relatively uncommon in rose (Rowley 1960a; Shahare and Shastry 1963) and may be due to greater viability of euploid gametes or chromosome elimination within aneuploid gametes or in the embryo after fertilization (Laurie and Bennett 1988; Rines and Dahleen 1990).

Triploidy and modern rose cultivars

Triploids were found among more than half of the horticultural classes (**Tables 2, 3**) and were especially common among popular, award winning shrub roses marketed for low-maintenance landscape use. Polyploidization can alter plant morphology (Semeniuk and Arisumi 1968; Basye 1990; Ma *et al.* 1997; Kermani *et al.* 2003; Zlesak *et al.* 2005). Among induced tetraploids of *R. chinensis minima*, Zlesak *et al.* (2005) found generally larger and darker green leaves and stems, thicker foliage and petals, and less branching relative to diploids. In addition, the growth rate of somatically-induced tetraploids reported by Zlesak *et al.* (2005) was generally less than that of diploids (pers. obs.). Triploidy may be a favorable balance between traits generally associated with tetraploidy and diploidy for roses used as landscape shrubs. Traits such as increased branching, dense growth habit, high overall growth rate, and copious bloom production for color effect are valuable for this market and are more likely to be favored at lower ploidy levels. Larger flowers and heavy petal substance for increased longevity are more likely to be favored at higher ploidy levels, desirable features for especially the cut flower market. In addition, triploids generally have reduced fertility relative to diploid or tetraploid roses (Rowley 1960b; Leus 2005) which can facilitate reduced fruit set without manual removal of spent flowers and faster reflowering. It is unclear to what extent the trend toward triploidy in landscape roses is a conscious breeding objective or an unintentional by-product of trait selection.

Triploid roses can be generated from crosses of any parental ploidy combination involving diploid, triploid, or tetraploid parents (**Table 4**) (El Mokadem *et al.* 2002a, 2002b; Barden and Zlesak 2004; Leus 2005). Meiosis can be quite variable in modern rose cultivars and include heteromorphic pairing and varying rates of univalents, bivalents, and multivalents (Erlanson 1933; Shahare and Shastry 1963; Ma *et al.* 2000). Variable homology between homologous chromosomes of contributing species genomes within modern roses, translocations, and duplications may lead to meiotic abnormalities and gametes of variable chromosome number. Perhaps the variable and often low fertility found particularly in polyploid rose cultivars (Shahare and Shastry 1963) may be associated with roses being an asexually-propagated crop. Since roses are perennial and cultivars are asexually propagated, they can be perpetuated indefinitely for continued attempts at hybridization, even if fertility is low. This has contributed to the continuation of cultivated roses with wide variability for meiosis and fertility (Shahare and Shastry 1963: Leus 2005). For other, especially annual crops, strong selection pressure is imposed for fertility and fecundity, traits often associated with orderly meiotic patterns and euploid gametes.

2*n* and 4*n* gametes

Mechanisms of 2*n* gamete formation have only recently been reported in a group of diploid rose genotypes primarily derived from tetraploid cut flower cultivars, and the inheritance of these mechanisms has yet to be determined (Crespel *et al*. 2002; El Mokadem *et al*. 2002a, 2002b; Crespel *et al*. 2006). Although the inheritance of mechanisms leading to 2*n* or 4*n* gametes in roses have not been reported, clones producing 2*n* gametes can often be traced back to parents that also produce 2*n* gametes. For instance, 2*n* pollen is produced by breeding line 1W13, an open-pollinated seedling of 1G84 ('Orange Honey' x 4BA3). Breeding line 4BA3 is a diploid polyantha genotype which has been identified as producing 2*n* pollen in a previous study (Zlesak *et al*. 2005). In addition, some 2*n*-pollen-producing diploids were derived from tetraploid cut flower roses that also produced 2*n* pollen, such as 'MEIhelvet' (aka 'Sweet Promise') which was also found to produce 2*n* pollen in this study (Crespel and Gudin 2003).

Rosa woodsii-2, a hexaploid, produced 4*n* pollen. *Rosa woodsii* has only been reported to occur as diploid (Cairns 2000). This 6*x* clone was found among a stand of *R. woodsii* growing near Naches, Washington (USA) by Joan Monteith and shares morphological similarities with other members of the stand. Rooted cuttings of *R. woodsii* clones neighbouring *R. woodsii*-2 were obtained and root tip squashes revealed they were diploid (unpublished data). *Rosa woodsii*-2 may have resulted from the union of 2*n* and 4*n* gametes generated by diploid *R. woodsii* and may have inherited the ability to generate 4*n* pollen. Typically, mechanisms governing 2*n* or 4*n* gametes in plants are controlled by a major locus in the homozygous recessive state (Mok and Peloquin 1975; McCoy 1982), but dominant inheritance of 2*n* gametes is also possible (Ortiz 1997).

Gametes which are 2*n* can transmit high levels of parental heterozygosity to progeny, especially if they arise from first division restitution mechanisms (Hermsen 1984; Peloquin *et al*. 1989; Crespel *et al*. 2002). Meiotic polyploidization relative to somatic polyploidization led to greater vigor among progeny in potato, another primarily outcrossing crop (Tai and De Jong 1997). The rate of 2*n* gamete production of clones having the capacity to produce them can be influenced by environment, and the frequency of 2*n* pollen can be increased using recurrent selection (McHale 1983; Parrot and Smith 1986; Ortiz and Peloquin 1992). In roses both first and second division restitution mechanisms of 2*n* gamete formation have been identified or are suspected, respectively (Crespel *et al*. 2002; El Mokadem *et al*. 2002a; Crespel *et al*. 2006). The identification of 2*n* or 4*n* pollen among diploid, tetraploid, and hexaploid genotypes in this study offers additional germplasm from which to study 2*n* or 4*n* pollen formation and highlights additional cultivars producing such pollen that are readily available to breeders.

CONCLUSION

This study demonstrates that pollen diameter in rose is a useful tool to predict sporophytic and gametophytic ploidy levels, especially in a breeding program like that of the author which utilizes diverse germplasm, and expands the findings of previous authors (Erlanson 1931; Lewis 1957; Jacob and Pierret 2000). Unfortunately, guard cell length proved not to be very useful for widespread ploidy prediction across divergent germplasm, even though it has utility in specific situations like the *R. carolina* complex (Joly *et al*. 2006) and in polyploidization studies. In order to best interpret pollen diameter data, familiarity with rose taxonomy is useful in order to recognize roses in or recently derived from especially section *Caninae* as well as species relationships and polyploidization events such as in the *R. carolina* complex. Pollen volume was demonstrated to be highly associated with pollen ploidy and points to great utility of pollen diameter for predicting paternal ploidy contribution. Pollen diameter is also useful for sporophytic ploidy prediction, although there are complications limiting its utility, as with other indirect ploidy assessments like flow cytometry. Pollen diameter can be an especially useful tool to study meiotic polyploidization. In addition, pollen diameter should be able to help breeders skew offspring to desired ploidy level(s) by choosing more amenable parents and even sorting and utilizing pollen of desired size with tools such as flow cytometry (Leus 2005) or appropriately sized nylon mesh (Eijlander 1988; Okazaki *et al*. 2005).

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