The Discovery and Naming of the Cascade Strawberry
(*Fragaria cascadensis* Hummer)

Kim E. Hummer, USDA ARS NCGR
33447 Peoria Road, Corvallis, Oregon 97333-2521, Kim.Hummer@ars.usda.gov

I have a great job. I am the Research Leader and Curator of a US Department of Agriculture *ex situ* (off site) genebank and research laboratory called the National Clonal Germplasm Repository (USDA ARS NCGR). At this facility in Corvallis, Oregon, my staff and I preserve about 12,000 living plants representing collections of important specialty crops, including berries, pears, hazelnuts, mint, hops, and their wild relatives.

Our assignment is to collect, maintain, distribute, and evaluate the global genetic diversity of these crops. We're part of the US National Plant Germplasm System. The federal government does good work in germplasm[1] conservation through our program!

Type specimen of *F. cascadensis*. If you look closely, white hairs are visible on the upper leaf surface of the four-foliate leaf. Note that the distal tooth of the distal leaflet is smaller than that of adjacent teeth. Photo by author

Our facility maintains plants in the field in orchards, in containers in greenhouses and screenhouses, as tissue-cultured plantlets, and as seed. We preserve heritage cultivars and species of crop wild relatives. Each year we host the public at open house events, in which visitors sample fruit of the 350 blueberry genotypes in mid-July, and about 2,000 types of pears in late August. At other times, the public can visit our laboratory by appointment.

As curator of the strawberry collection, I have had the good fortune to have participated in more than 18 major international plant-collecting expeditions throughout the world to obtain samples of regional cultivars and diverse wild species for our genebank. Our strawberry collection includes 42 taxa (including species and subspecies) and 1,842 accessions from 42 countries.

Native strawberries in Oregon

Until recently, only four native taxa of *Fragaria* were known in Oregon: Virginia strawberry (*Fragaria virginiana* ssp. *platypetala*), alpine or woodland strawberry (*F. vesca* ssp. *bracteata*), beach strawberry (*F. chiloensis*) and a natural hybrid between beach and Virginia strawberries (*F.* × *ananassa* nothosubsp. *cuneifolia*). Like many plant species, ploidy levels[2] vary among *Fragaria* species. The base number of chromosomes in a haploid strawberry gamete is $x = 7$ (Darrow 1966). Alpine strawberry, Oregon's only diploid strawberry, is widespread, occurring in the Coast Range, the Willamette Valley, the Cascade Mountains, and throughout eastern Oregon in moist montane habitats. The octoploid beach strawberry, with two subspecies recognized by some authorities, is found along the coast. The octoploid Virginia strawberry, which was named for the Virginia colony in the 1700s, is widespread throughout the United States; in Oregon, it is found from the western Cascade foothills and eastward in moist montane habitats. The natural strawberry hybrid, which occurs in the Coast Range and the Willamette Valley, is also octoploid, like its parents.

As everyone knows who has ever grown strawberries in their garden or a container, these plants reproduce vegetatively by runners as well as by seed. The seeds are found in the achenes on the surface of the tasty fruits. Wild populations of strawberries consist of clonal colonies of plants with either imperfect (male or female) or perfect (hermaphrodite) flowers arising from the runners. Hermaphrodite plants are very rare in the species *F. chiloensis*, and this species is considered dioecious. In populations of *F. vesca* subsp. *bracteata* only females and hermaphrodites are found, a situation called gynodioecious (Staudt 1999, Tennessen *et al.* 2013).

1 The term germplasm refers to living tissues that contain the genetic makeup or propagules of plant species.

2 Polyploid organisms have two or more sets of homologous chromosomes, designated as 4x, 6x, etc.

A puzzle in strawberry polyploidy

During a routine survey of wild strawberry germplasm using molecular markers, our geneticist, Dr. Nahla Bassil, and her graduate student, now Dr. Wambui Njuguna Young, found some anomalies in some samples of what we thought were the octoploid Virginia strawberry. They observed several samples with unusually high numbers of alleles per locus, many more than they expected. This discovery prompted us to count the chromosomes of those samples using a microscope. Visiting scientists Dr. Tomohiro Yanagi from Japan, Dr. Preeda Nathewet from Thailand, and I found that the atypical samples did, in fact, have more chromosomes; they were decaploid (10*x*), not octoploid (8*x*). Now this was really intriguing, because the only previously known naturally occurring decaploid strawberry grows on the Kurile Islands between Japan and Russia (Hummer *et al.* 2009). Other known decaploids are cultivated types that were produced in a laboratory, not found in the wild.

Inflorescences of the Cascade strawberry showing young and mature fruits. Photo by author.

Tracking decaploid strawberries in the mountains of Oregon

One of the plant samples in question was collected in 1982 by the germplasm repository's former curator, Dr. Otto Jahn along the Pacific Crest Trail near Big Lake, south of Santiam Pass in eastern Linn County, Oregon (ca. 1400 m. elev.). I became curious whether this decaploid cytotype[3] was just an anomaly or whether other strawberry plants nearby had similar chromosome numbers. Also I wondered if decaploid plants were widely distributed or whether there was just one small colony. No one had described a native wild decaploid strawberry from North America before, although many strawberry breeders had synthesized them in the laboratory using chemicals and specific crosses.

So I set out to obtain samples of wild strawberries from many locations around Oregon. I first went to the original location along the Pacific Crest Trail and obtained samples from east and west of Big Lake (near Hoodoo Butte). The strawberries were growing alongside a forest road and in the alpine meadows.

We started analyzing the number of chromosomes of the strawberries using a technique called flow cytometry, which is faster than counting chromosomes using a microscope. The first results were exciting: many clones near the original locality were decaploid. Soon I found that many sites from elevations above 1,000 m. in the Cascades, starting near Mt. Hood in the north and extending to Crater Lake in the south, supported decaploid strawberries. Like other *Fragaria* taxa in Oregon, plants from these populations are sub-dioecious.

One location where *F. cascadensis* grows in the high peaks of the Oregon Cascades near the Pacific Crest Trail. Photo by author.

3 An organism with a chromosome number that differs from others.

Identification key for wild *Fragaria* in Oregon

1a. Leaves thick (or moderately so), lower surface often strongly reticulate-veined, upper surface dark green or bluish green; achenes large to medium; plants coastal to inland.

2a. Leaves thick, strongly reticulate-veined beneath, upper leaf surface dark green; achenes 1.8 (1.4-2) mm long; plants coastal, octoploid (2n=8x=56); beach strawberry. ..... *F. chiloensis*

3a. Petioles, peduncles, pedicels and runners with appressed-ascending hairs, occasionally macroscopically almost glabrate ..... subsp. *lucida*

3b. Petioles, peduncles, pedicels and runners with spreading, dense hairs ..... subsp. *pacifica*

2b. Leaves somewhat thick, not strongly reticulate-veined beneath, sometimes bluish green to slightly glaucous; achenes 1.4 (1.3-1.75) mm long; plants coastal to inland, octoploid (2n=8x=56); hybrid strawberry..... *F. ×ananassa* nothosubsp. *cuneifolia*

1b. Leaves thin, not reticulate-veined beneath, upper surface bluish green; achenes 1.4-1.6 mm long; plants inland.

4a. Leaves bright green; leaflets ovate or obovate to slightly rhomboidal; distal tooth of terminal leaflet usually longer than adjacent teeth; teeth ca. 38 or more per leaf; flowers ~ 20 mm in diameter; inflorescence usually above foliage; flowers usually perfect, sometimes female; calyx mostly reflexed from ripe fruit; achenes 1.4-1.6 mm long, frequently with persistent style; Coast Range, Willamette Valley, Cascade Mountains, moist montane habitats in eastern Oregon; plant diploid (2n=2x=14); alpine or woodland strawberry ..... *F. vesca* subsp. *bracteata*

4b. Leaves green to bluish green; distal tooth of terminal leaflet usually shorter than adjacent teeth; inflorescence usually lower than foliage or variable length; flowers male, female, or perfect; calyx mostly clasping ripe fruit; achenes 1.5 (1.3–1.8) mm, style not persistent.

5a. Upper leaf surface glabrous; achenes generally tear-drop shaped, about 1.5 mm long; Oregon Coast Range, Willamette Valley eastward in moist montane habitats throughout Oregon, generally < 1,000 m elev.; plants octoploid (2*n*=8*x*=56); Virginia strawberry ..... *F. virginiana* subsp. *platypetala*

5b. Upper leaf surface with scattered white hairs (~ 1mm); many achenes comma-shaped with concave edge, sometimes tear-drop shaped; crest of Cascade Range from ca. Mt. Hood to Crater Lake, extending down west side to about 1,000 m elev.; plants decaploid (2*n*=10*x*=70); Cascade strawberry ..... *F. cascadensis*

Achenes of *F. vesca* subsp. *bracteata*, *F. cascadensis*, and *F. virginiana* ssp. *platypetala.* (Images by Dr. Sugae Wada).

(2*N* = 10*x* = 70) chromosomes of the Cascade strawberry, *F. cascadensis*. The bar = 5 µm. Image by Dr. Preeda Nathewet.

Should the decaploid plants be considered a new species?

After establishing that the decaploids were widespread along the crest of the Cascades, I began collecting samples from many river drainages and trails in the Willamette Valley to see how far west the distribution of the decaploids extended. Samples from the Valley and the Coast Range proved to be octoploids. It occurred to me that perhaps the decaploids were restricted to higher elevations, so I sampled strawberries on Marys Peak (ca. 1240 m.). Not so! Samples from Marys Peak were octoploid, as were populations in eastern Oregon. Samples from high elevations in the Blue and Wallowa Mountains, the Washington Cascades, and in California from Mount Shasta and Mount Lassen were also octoploid. Thus, the decaploid strawberry was limited, as far as we know, to a band high in Oregon's Cascade Range.

The restricted distribution and the higher chromosome number, which served as a barrier to crossing with the octoploid Virginia strawberry, signified that these populations might represent a new species. I asked my colleague, Dr. Aaron Liston (Professor and Director of the Oregon State University Herbarium), what it took to name a new species. He said I would first need to determine how the morphology of the decaploids differed from other Oregon strawberries. Then I would need to write and publish a detailed description based on a type specimen[4], along with a taxonomic key to separate it from other Oregon species of *Fragaria*.

4 A type specimen is the plant, deposited in a herbarium, on which the description and name of a new taxon is based.

Naming the new species

I went to one of my favorite populations of the decaploid strawberries, at the entrance road to Waldo Lake (1700 m.) in Lane County in the western Cascades. I began systematically measuring and counting flower parts (anthers and petals), leaves, and runners, making notes of any unusual characters. At first, I did not detect differences in morphology between the decaploid and the octoploid plants at other locations.

Then, one day, I re-read a description of *F. virginiana* subsp. *platypetala* written by Dr. Guenter Staudt, a German taxonomist, in his monograph on strawberries of North America (Staudt 1999). He described the upper leaf surface of Virginia strawberry as being "smooth and usually glaucous." I looked at the leaf of a decaploid under a microscope and observed that it had a number of 1 mm. long white hairs on the adaxial (upper) surface; it was not "smooth." Those hairs became my first diagnostic character. As I looked at more samples, I confirmed the distinction: those with hairs on the upper side of the leaf occurred consistently on decaploid samples; the ones that were bald on top were octoploid.

Then I began to recognize differences in achenes: edges of decaploid achenes curved, while edges of octoploid achenes were straight. The achenes of the diploid alpine strawberry are smaller than the others and have persistent styles. While this species also has hairs on the upper leaf surfaces, it could be separated from the decaploids by its prominent veins and characteristics of the teeth on leaflet margins.

Having pinpointed key morphological differences, it was time to review the strawberry collection at the Oregon State University Herbarium. As I anticipated, I found a number of specimens labeled Virginia strawberry (some annotated by Staudt!) with 1 mm. hairs on the upper surface of the leaves, all from the high peak region of the Oregon Cascades (except for one 1915 collection with only the vague description "Hood River").

Now I had a distribution map, morphological differences, and differences in the chromosome number. I chose a population near Waldo Lake east of Oakridge in Lane County for the type locality[5] and published an article describing the new species, naming it for the Cascade Range of Oregon, *F. cascadensis* (Hummer 2012).

Populations of this new species have been found from near Mount Hood on the Burt Lake Trail, Echo Mountain Trail, near Hoodoo Butte, Hayrick Butte, around Big Lake, near Waldo Lake, near Diamond Lake, and to southwest of Crater Lake. Populations are found only above about 1,000 m. elevation on the west side of the crest in the Oregon Cascade Range. Significantly, all of the strawberries in this band of the High Cascades in Oregon were Cascade strawberry; no Virginia strawberries grow at this elevation.

Male (top), female (middle), and hermaphroditic flowers (bottom) of the Cascade strawberry. Photos by author.

5 The type locality is the place where a type specimen was collected.

NCGR screenhouses in Corvallis. Photo by author.

Strawberry collection at NCGR. Photo by author.

How might the decaploid Cascade strawberry have formed?

During the past century, scientists have created decaploid strawberries in the laboratory using colchicine, a chemical mutagen, but the first discovery of a native wild decaploid (10*x*) species was of *F. iturupensis* Staudt from the Kurile Islands (Hummer *et al.* 2009). The exact process that produced this decaploid isn't known, but mutations causing the spontaneous doubling of chromosomes in gametes may occur naturally following hybridization. Such mutations occur fairly frequently in strawberry pollen, in which the genetic material for two pollen grains gets packaged into a single large one. For example, colonies of plants with mixed ploidy levels (5x, 6x, 9x) have been identified in California as a result of crossing of diploid (*F. vesca* ssp. *californica*) and octoploid (*F. chiloensis*) strawberries (Bringhurst 1990).

This same process could have occurred in Oregon for Cascade strawberry. To fully understand how the decaploid plants evolved, plants with intermediate ploidy levels between 2*x* and 10*x* would need to be identified within or near the decaploid populations. It might be that the intermediate-ploids necessary to form the decaploid may have had a transient existence in Oregon. Many intermediate ploidy levels have very low fecundity because their homologous chromosomes do not pair properly during meiosis. However, vegetative reproduction of strawberries by runners may have allowed unstable intermediate forms to persist long enough to produce a stable decaploid.

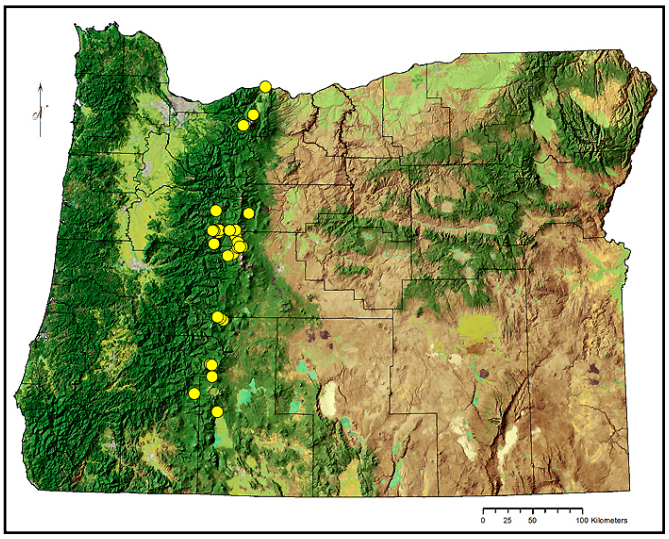
An explanation for the distribution of Cascade strawberry?

Scientists examining the chloroplast genome of the octoploid Virginia strawberry determined that it evolved between 400,000-2 million years ago (Njuguna *et al.* 2012). Thus, if the decaploid Cascade strawberry originated from a combination of the genomes of Oregon's octoploid Virginia and diploid alpine strawberries, it must have evolved more recently. This led us to consider events in Oregon that might have fostered a narrow band of strawberries in the Oregon High Cascade Range. About 10,000 years ago, Pleistocene glaciers retreated northward, leaving only a remnant ice cap over the highest part of the Oregon Cascades (Porter *et al.* 1983). A subsequent hypsithermal (rapid warming) event melting this ice cap would have opened up prime territory for expansion of a newly formed decaploid strawberry species with a pioneering nature.

Potential of the decaploid Cascade strawberry for commercial use

If trends over the past decades are an indication, consumers are interested in ever bigger strawberries. Fruit of the cultivated hybrid octoploid strawberry is much bigger than that of the diploid alpine strawberry. Thus, one might predict that the decaploid Cascade strawberry fruit would be even larger. Unfortunately, its fruit diameter is small, about the size of a thumbnail. Fruits of the common commercial strawberry (cultivars of octoploid *F.* × *ananassa*) weigh ten times that of the Cascade strawberry. Moreover, the flavor of the decaploid fruit is mostly bland and not as complex as that of the alpine strawberry.

Despite this, breeders may want to use it in developing a new class of cultivated strawberries at the decaploid level. Several artificial decaploid strawberries (Dermen and Darrow 1938) already exist: in Germany, *F.* ×*vescana* hybrids from polyploid



Distribution map of the Cascade Strawberry from the Oregon Flora Project, (accessed 3 December 2014) published with permission.

Insect (hover-fly, possibly *Sphaerophoria sulphuripes*) visiting a male flower of Cascade strawberry. Photo by author.

F. vesca and cultivated *F.* × *ananassa*; in Japan, hybrid polyploid aromatic F. *nilgerrensis* with cultivated *F.* × *ananassa.* Perhaps new flavors or resistant genes could be available to agriculturalists by intercrossing native and artificial decaploid strawberries. Scientists from around the world are now examining the genetics of the decaploid strawberries.

Oregon's fifth native strawberry in the wild

So, now Oregon has five native strawberry species, not four. My team at the strawberry genebank and I will continue to collect samples to see if any strawberries with intermediate ploidy can be found in Oregon. That will help us determine how the Oregon decaploids strawberries came to be.

While hiking in the Cascades, I found that I wasn't the only one interested in the decaploid flowers. Several different types of insects, including flies and ants, visited the flowers as well. Maybe the fly pollinators played a role in the evolution of this strawberry species. Research on the entomology of the pollinators of the High Cascades is underway by Dr. Andrew Moldenke in the Department of Botany and Plant Pathology at Oregon State University.

The next time you are hiking near the Pacific Crest Trail in the Oregon Cascades, look down and see if the strawberry plants at your feet have green-blue leaves. Feel for hairs on the top side of the leaf surface or look closely at the leaves with your hand lens. If you find hairy leaves, you have likely found Oregon's newest strawberry species, the Cascade strawberry.

References

Bringhurst R. 1990. Cytogenetics and evolution in American *Fragaria*. HortSci. 25(8):879-881.

Darrow G. 1966. The Strawberry. New York (NY): Holt, Rinehart and Winston.

Dermen H, Darrow GM. 1938. Colchicine-induced tetraploid and 16-ploid strawberries. Proc. Am. Soc. Hort. 36:300–301.

Hummer K, Nathewet P, and Yanagi T. 2009. Decaploidy in *Fragaria iturupensis* (Rosaceae). Am. J. Bot. 96(3):713-716.

Hummer K. 2012. *Fragaria cascadensis* Hummer. J. Bot. Res. Inst. Texas 6(1):9-15.

Njuguna W, Liston A, Cronn R, Ashman T, Bassil NV. 2012. Insights into phylogeny, sex function and age of *Fragaria* based on whole chloroplast genome sequencing. Molec. Phylogenet. Evol. http://dx.doi.org/10.1016/j.bbr.2011.03.031.

Porter SC, Pierce KL, Hamilton TD. 1983. Late Wisconsin mountain glaciation in the Western United States. Pp. 71-111 in Late-Quaternary Environments of the United States, Vol. 1. Minneapolis (MN): Univ. Minnesota Press.

Staudt G. 1999. Systematics and geographic distribution of the American strawberry species: Taxonomic studies in the genus *Fragaria* (Rosaceae:Potentilleae). Publ. Bot. 81. Berkeley (CA): Univ. Cal. Press.

Tennessen JA, Govindarajulu R, Liston A, Ashman T-L. 2013. Targeted sequence capture provides insight into genome structure and genetics of male sterility in a gynodioecious diploid strawberry, *Fragaria vesca* ssp. *bracteata* (Rosaceae). *G3* (Bethesda). 3(8):1341-51.

Kim E. Hummer was born in Washington, DC in 1952, received her BS in Biology from St. Lawrence University in 1974, her MS in plant and soil science from the University of Vermont in 1978, and her doctorate in horticulture from Oregon State University in 1981. Her scientific expertise includes the conservation of fruit, nut, and specialty crop genetic resources. Her present research passion involves the study of ploidy in berry species. She also actively studies genetics and chemical constituents of strawberries, blueberries, blackberries, raspberries, currants, gooseberries, and unusual berry crops such as blue honeysuckle.

During her career she has participated in more than 18 plant collecting expeditions to locations including Canada, China, India, Italy, Japan, Portugal, Russia, and throughout the United States including Alaska and Hawaii. She was selected as Specialty Crop Curator for the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository in Corvallis, Oregon in 1987 and became Research Leader of that gene bank in 1989. Dr. Hummer is an active member of the American Society of Horticultural Science, and was selected as a Fellow in 2006. She was the first woman president of the American Pomological Society (2004-2006) and, in 2006, chaired the committee that developed the Global Conservation Strategy for Strawberry, sponsored by the Global Crop Diversity Trust. From 2002-2010, she was Chair of the International Society for Horticultural Science, Commission on Plant Genetic Resources, and was the first woman Vice President of that Society from 2010-2014.