THE WORLDWIDE SIGNIFICANCE OF KARYOTYPIC VARIATION IN *ROSA ACICULARIS* (ROSACEAE)

Walter H. Lewis and Memory Elvin-Lewis

Biology Department, One Brookings Drive, Box 1137
Washington University in St. Louis, Missouri 63130-4899, U.S.A.
The Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63110-0299, U.S.A.
wlewis@wustl.edu elvin@wustl.edu

ABSTRACT

Evidence is provided which shows that there is a wider variation in karyotypes of *Rosa acicularis* subspecies seen within their ranges in North America (*Rosa acicularis* Lindl. subsp. *sayi* (Schwein.) W.H. Lewis and the Eurasian species (*Rosa acicularis* Lindl. subsp. *acicularis*) than was originally presumed. It is proposed that the evolution and distribution of these karyotypes into two distinct subspecies occurred following the closure of the Turgai strait when the climate radically changed within both continents. Their distributions are similar in that they are not only dependent upon the latitudes where they are found but on the ecology and climate that supports their growth. Parallel ethnobotanical and medicinal uses are also evident as is their value in horticulture, where especially in North America their genes for cold hardiness are being exploited. Concerns regarding how global warming may affect these various karyotypes are also discussed.

RESUMEN

Se proporciona la prueba que muestra que hay una amplia variación en cariotipos de la subespecie de *Rosa acicularis* vista dentro de su gama en América del Norte (*Rosa acicularis* Lindl. subsp. *sayi* (Schwein.) W.H. Lewis y las especies euroasiáticas (*Rosa acicularis* Lindl. subsp. *acicularis*) que originalmente fue presumida. Se propone que la evolución y distribución de estos tipos de poliploidía en dos subespecies distintas se produjeron tras el cierre del estrecho de Turgai cuando el clima cambió radicalmente en ambos continentes. Sus distribuciones son similares en que no son sólo depende de las latitudes donde son encontradas, sino también sobre la ecología y el clima que apoya su crecimiento. Usos paralelos etnobotánicos y medicinales también son evidentes como su valor en horticultura, donde especialmente en América del Norte se explotan sus genes para la resistencia al frío. También se discute la preocupación por el calentamiento global y cómo puede afectar a estos cariotipos diferentes.

INTRODUCTION

Rosa acicularis Lindl. has the most extended native range of any species in the genus *Rosa* (Lewis 1959) and is the only rose in the world that is circumpolar or global in distribution (CYSIP 2016). It is referred to as the Arctic or Circumpolar rose (Shepherd 1978) and is represented by two subspecies found in either Eurasia (*Rosa acicularis* ssp. *acicularis* Lindl.) or North America (*Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis), which not only vary in their geographic locations but also in at least nine morphological characteristics and the ploidy levels which predominate (Watson 1885; Hurst 1929; Erlanson 1930; Lewis 1959).

The presence of both subspecies in Alaska suggests that the species most probably utilized the Bering Land Bridge during the Eocene when genetic exchanges between the two continents were possible. Called Beringia, this ice-age connection of grassland steppe between West Siberia and Alaska was estimated to be at least one thousand miles wide. The climate was very different from today, with temperatures not unlike the temperate to warm temperatures which sustain many roses growing worldwide. It is believed that as the temperature became cooler when the Turgai strait closed roses began to migrate south and west towards Europe and south and east in North America (Fougère-Danezan et al. 2015). It is possible that during this period the evolution of the two subspecies *Rosa acicularis* Lindl. took place resulting in the development of a polyploid series on both continents. Those remaining in the higher latitudes of the colder north began to produce multiple copies of their chromosomes e.g., $2n = 56$ to survive, with others evolving specific karyotypes ($2n = 42$; $2n = 28$) to accommodate the climate and ecological niches they inhabited.

Data on *Rosa acicularis* Lindl. presented here attests to this possibility since ploidy levels of its two subspecies are clearly relevant to its survival within the range of their distribution in North America and Eurasia. In both continents it is evident that the environment and ecology in which they are found have influenced

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these variations. These observations merit a more detailed discussion than what is already found in earlier publications (Erlanson 1938; Lewis 1958, 1959; Love et al. 1971) or as reported in the *Flora of North America* (Lewis et al. 2015). This information should be particularly useful to scientists wishing to understand the impact of the environment on the genetics of an organism as well as to hybridizers that are considering using this species in the development of new horticultural varieties.

ECOLOGY AND DISTRIBUTION OF *ROSA ACICULARIS*

Rosa acicularis Lindl. has a wide ecological range in Eurasia and can be found in forests and steppes, ascending to the lower part of the sub alpine zone in high mountain ranges in addition to extending into the lowland tundra. Usually the shrub may be found alone or in small groups and only rarely occurs in denser populations. In forests it grows in the undergrowth where some light penetrates. In southern locations it prefers habitats that are shady and damp in deciduous forests adjacent to the steppes as well as growing on river flood basins. In northern regions it prefers river banks formed by limestone, slate, marls and sandstone rather than damp and cold soils. It can be located on southern slopes and river valleys, and particularly in Eurasia penetrating the forest-tundra and tundra. (Yuzepchuk 1941; Sokolov et al. 1980; Koropachinskiy & Vstovskaya 2002; Chuhina & Bagmet 2004).

In Eurasia, it grows wherever spruce, pine and birch forests predominate in Sweden, Norway and the Finnish taiga and north and north-east of the European part of Russia (Karelia, Murmansk), in the Volga region and Ural mountains. It is also found, within forests of spruce, pines or larches including the vast larch woodlands of eastern Siberia, and the Eastern and Western Russian Federations of Siberia and the Russian Far East (Primorye, Kamchatka). As the taiga extends southward to areas of northern Kazakhstan, Kyrgyzstan, and the Far East of northern Mongolia, and northern Japan the species has been located growing in the Tuva Republic of the Russian Federation, Outer Mongolia, Saur, Tarbagatai, Dzungarian Ala Tau, northern China (Gansu, Hebei, Heilongjiang, Jilin, Liaoning, Nei Monggol, Shaanxi, Shanxi, Xinjiang), Korea and Japan (Hokkaido, Honshu). Like its habitat on the North American prairies, it may also be located growing on adjacent steppes (Lewis 1959; Yuzepchuk 1941; Koropachinskiy & Vstovskaya 2002; Sokolov et al. 1980; Yu et al. 2014U.S. National Plant Germplasm System, *Rosa acicularis*, 2007). Additional studies of the Northern Altai-Sayan mountains indicate it is also present in the forest steppe belts and dry hemiboreal forests of the region (Riznicková et al. www.terrestrial-biozones.net/Holarctic, accessed July 2017). The distribution map based on herbarium collections and the literature by Chuhina and Bagmet (2004) beautifully illustrates its distribution throughout Eurasia.

In central Alaska and the Yukon, the subspecies is more likely to resemble the Eurasian *Rosa acicularis* subspecies *acicularis* Lindl. with some hybrids or introgressions possible with *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis) (Lewis 1959; Hultén 1974). The range of the latter subspecies extends within the North American taiga, referred to as boreal or snow forests, from the Arctic Circle and southward throughout parts of North America as well as to its prairies and Great Plains (Crane 1990). Several websites such as the Flora of North America (Lewis et al. 2013) and USA-NPN (2016) illustrate its distribution by State or Province in North America. For a map of global distribution, which also illustrates morphological variations within the subspecies reference to the monograph of Lewis (1959) is recommended. A detailed description of the ecology and climate of steppe localities as well as outline maps of steppe regions in Eurasia and North America is provided by Kintgen (2017). Descriptions of the tiaga or boreal forests including their global distribution maps can be elicited online e.g., www.blueplanetbiomes.org/taiga.html.

In Canada it is found throughout the Yukon and Northwest Territories and Nunavut and the Provinces of Canada from British Columbia East of the Coastal Mountains extending through Alberta up to 6550 ft., Saskatchewan, Manitoba, Ontario and New Brunswick (Crane 1990). It prefers alluvial soils found on the Saskatchewan (Dirschi & Coupland 1972) and McKenzie river deltas (Pearce et al. 1988). In Alberta it has been found to be moderately tolerant of crude oil and can be resistant to drought on amended sands with oil tailings (Watson et al. 1980). Because of lack of light it is infrequent in dense black spruce forests (*Picea mariana)* but commonly occurs among white spruce (*Picea glauca*) forests and as they merge into the northern hardwood forests of cottonwood (*Populus* spp.), quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) and south of the tree line to the grass lands of the prairies (Crane 1990).

In the Northern Rockies of both Canada and the United States it can be found in the undergrowth or adjacent to forests of Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*, ponderosa pine (*Pinus ponderosa*), Douglas fir (*Pseudotsuga menziesii*) and subalpine fir (*Abies lasiocarpa*) (Crane et al. 1983; Reed 1976; Steele et al. 1983).

Similarly in the United States it grows primarily in the northern states or in mountainous regions wherever cool boreal forests exist, and in some states can also extend onto the northwest Great Plains. In Alaska it prefers well drained alluvial soils and river deltas but is susceptible to rapid freezing and thawing on gravels that are low in nutrients (Dyrness & Grigal 1979). It can be found in aspen forests associated with old burns, with willows (*Salix* spp.), alder (*Alnus* spp.) and high bush cranberry (*Viburnum edule*) and at tree line in thickets, bogs and alongside roadways (Dyrness & Viereck 1979). It grows in the Rocky Mountains, Wyoming Basin, Black Hills uplift, Upper Missouri Basin and Broken Lands and because of its moderate shade tolerance, and depending upon the locality, it can dominate in the understory of deciduous trees such as aspen (*Populus* spp., paper birch (*Betula papyrifera*), basswood (*Tilia* spp.), sugar maple (*Acer saccharum*) and with conifers such Jack pine (*Pinus banksiana*), ponderosa pine (*Pinus ponderosa*) white spruce (*Picea glauca*), black spruce (*Picea mariana*) and Engelmann spruce (*Picea engelmannii*), subalpine fir (*Abies lasiocarpa*) and Douglas fir (*Pseudotsuga menziesii*). Its presence has been recorded in North Central U.S.A.—Illinois, Iowa, Minnesota, North Dakota, South Dakota, Northern Wisconsin, the North East—Maine, Massachusetts, Michigan around lake dunes, New Hampshire, New York, Vermont, the Northwest - Colorado up to 10,900 ft., Idaho, Montana up to 9,000 ft, Wyoming up to 10,900 ft, and Washington and the South-Central region of New Mexico. Within some of these regions such as the Minnesota forests it prefers open spaces to the canopy, around Lake Michigan is a seral dominant succession on lake dunes, and may be found in patches in the rough fescue (*Festuca scabrella*) grasslands of northern Montana along with serviceberry (*Amelanchier alnifolia*), and snowberry (*Symphoricarpos* spp.) (Lynch 1955). In areas surrounding the Great Lakes it can be found growing on gravel and sand along the shoreline, within nearby woodlands with Jack pine (*Pinus banksiana*) and Oak (*Quercus*) and in openings of conifer forests as well as along rocky ridges, in moist thickets and swamps (Voss 1985). Throughout its distribution it can be a pioneer on river gravel bars, wherever mineral soil is exposed, and may be the first to appear after forest fires or after logging (Watson et al. 1980) emerging from its unaffected stems and root stocks or as seedlings (Crane 1990). For more details on these aspects and information regarding its historical nomenclature and conservation Schori (2003) provides an excellent and detailed review of this species in New England.

Rosa acicularis morphological diversity

It has been generally accepted, with the exception of a few Eurasian species found in Alaska (Lewis 1959; Hultén 1968) and where introgressions have been identified in north eastern Alaska and the Far East of Siberia, that these taxa are considered readily identifiable (Lewis 1959; Lewis et al. 2013) as are several morphological varieties known to specific regions in North America namely *R. acicularis* var. *bourgeauiana* Crép. ($2n = 42$) in the Alberta/Saskatchewan basin and according to Hultén (1974) is common in Alaska (Hurst 1928); *R. acicularis* var. *lacuum* Erlanson in Mackinac Co., Michigan and *R. acicularis* var. *rotunda* Erlanson in Cheboygan Co., Michigan (Lewis 2015). Also, a rare form with multiple petals (up to 15 petals) named *R. acicularis* Lindl., subsp *sayi* (Schwein.) Lewis f. *plena* was found in Moose Range Saskatchewan, Canada (Lewis 1958). Similar varieties are likely to occur throughout Eurasia such as those described for Russia: *Rosa acicularis* var. *gmelinii* (Bunge) C.A. Mey. (Meyer 1847); China: *Rosa acicularis* var. *glandulifolia* Y.B. Chang, Heilongjiang, Huma Xian, Muhe, north east forest 400 m, 11 Sep 1960, *S.Q. Nie 3548* (HT) (Chang 1981); *Rosa acicularis* var. *glandulosa* Liou. (Kitagawa 1979a); *Rosa acicularis* var. *pubescens* Liou. (Kitagawa 1979b); *Rosa acicularis* var. *setacea* Liou, Heilognjiang Forest. 2nd Sect 830. (lectotype: NEFI) *Xunke Xian* 27 May 1959 (Chang 1981); and Japan: *Rosa acicularis* var. *taquetii* (H. Lév.) (Nakai 1916).

Karotypes of *Rosa acicularis*, our global *Rosa*

The basic chromosome number of *Rosa* is 7, and multiples of this uneven number can be found throughout the genus and within some species (Ratsek et al. 1940). The latter is particularly true for *Rosa acicularis* Lindl. where its vast array of chromosome numbers represents an important cytogenetic mechanism towards the evolution of the species (Wood et al. 2009). Up until now it was generally considered that *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis, found in North America was primarily hexaploid (2*n* = 42) with only a few tetraploids (2*n* = 24) known, whereas the Eurasian subspecies *Rosa acicularis* ssp. *acicularis* Lindl. was essentially octoploid (2*n* = 56) with a few hexaploids (2*n* = 42) recognized (Lewis 1959). Also, the octoploids (2*n* = 56) found in Eurasia (Hurst 1928) were rare in North America and possibly only found in Alaska. Within this context it was accepted that higher ploidy levels were associated with their ability to survive under extreme growing conditions known to colder climates found in the higher latitudes of both continents (Lewis 1959; Erlanson 1929; Erlanson 1934; Hurst 1925).

MATERIALS AND METHODS

Rosa acicularis Lindl. ssp. *sayi* (Schwien.) W.H. Lewis specimens were collected in the field in Canada and the United States, with vouchers made and filed at the Missouri Botanical Garden (MO) and the University of British Columbia (UBC). Leaflets harvested at that time were immediately sent to Professor Anne Bruneau, Directrice de l'IRBV Directrice scientifique du Centre sur la biodiversité, University of Montreal, Quebec who conducted flow cytometry analyses on their DNA to determine their ploidy level. Cuttings were propagated under mist at Washington University greenhouses and were grown in the wild Rose garden established by the senior author in St. Louis, Richmond Heights, MO at his residence (38°37'11" N, 90°19'43" W, 533 ft). This locality is designated by the USDA as ecoregion 72f (River Hills), Palmer drought index 2, Hardiness zone 6a. The plants were grown in a semi-shaded area and surrounded by wired cages to protect them from rodent predators. They were observed throughout several growing seasons to see if they survived the USDA zone 6a climate (moderately moist, Northwest Prairie), which is characterized by an average low temperature of 45°F and a maximum of 66°F, 35.95 inches of precipitation, a growing season of 46–60 days > 86°F, the average first frost occurring from October 11–20th, winter temperatures which can reach below -5°F to -10°F and late frosts from April 1–10. During the late winter and early spring a series of warm periods followed by frosts, sometimes severe, are not unusual. Many early blooming species are negatively affected by these latter events, with buds being destroyed, or rosaceous fruit trees having crops diminished in yield (David Byrne, pers. comm. 2017). Whenever possible, herbarium vouchers collected over several growing season were made and are housed at MO. Root tips harvested after misting from our specimens and those harvested from rooted material received from Bruce A. Bennett Department of Environment, Government of Yukon in Whitehorse and growing in the Rose garden were fixed using "Farmer's fixative" e.g., a mixture of 95% ethanol and 25% glacial acetic acid at a ratio of 3:1 and sent to David Zlesak, Associate Professor of Horticulture, University of Wisconsin-River Falls for karotyping. Also specimens collected in Canada by the horticulturist Paul Olsen, frequently in collaboration with the rosarian Margit Schowalter were first sent to David Zlesak for karyotyping before potted specimens were forwarded to St. Louis for planting and study. Reference to numerous websites in 2016 was also necessary to determine hardiness zones, climate, e.g., world climate index (2007); World Maps of Köppen-Geiger climate classification, projected (1901–2100), Kottek et al. (2006), Rubel and Kottek (2010); Climatemps.com; USDA hardiness zone maps 1990, 2012) and information on geophysical and ecology of a particular region e.g., Encyclopaedia Britannica (2016); Royal Geographical Society (RGS). 2016 and the updated Köppen-Geiger climate map of the world (cited 2017).

RESULTS

North American Karyotype Distributions

Early studies showed that hexaploids (2*n* = 42) of *Rosa acicularis* Lindl. ssp. *sayi* were widespread and were found in Canada in Alberta, Saskatchewan, Manitoba and Ontario and in the US in Michigan, Colorado, Montana, Wyoming (Lewis 1959; Hurst 1928; Erlanson 1929; Erlanson 1930) as well as being present further south than was initially described by being identified both in southern Michigan (Erlanson 1934) and in the Ice Mountain area of West Virginia. In the latter instance, this discovery made by W.H. Lewis in 1954 was not surprising since these Mountains remain frozen year-round except for warming in a passage where *Rosa acicularis* ssp. *sayi* was found growing as if in its northern latitude. The specimen was collected in U.S.A. WEST VIRGINIA. Hampshire Co.: Ice Mountain, shaded path at base of mountain in a restricted area; 6 Jun 1954, *W.H. Lewis 2509* (MO), $2n = 42$ (Fig. 2.). This remains one of the species most south-eastern locations (Lewis 1957; Lewis 1959). Ice Mountain's current geolocation is approximately 39°21'48" N, 78°28'01" W. This site is unique and is considered a refugium from the last ice age for boreal species now only found in subarctic regions. This is because accumulated rock detritus at the base of the mountain possesses ice and ice vents causing a refrigeration effect in this temperate climate with hot summers and no dry season (Ice Mountain https://topics.revolvy.com/topic/Ice Mountain)(Kite, 2009).

Current additional studies have expanded the range of *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis hexaploids ($2n = 42$) throughout their ranges in Canada and the U.S.A. These collections have been subject to karotyping or flow cytometry, planted in the Lewis wild Rose garden, and observed over a number of growing seasons.

CANADA. ONTARIO. Algoma District: ca. 50 km N of Sault Ste Marie, Harmony Beach by Lake Superior, sand-loam soil in partial shade from trees and shrubs, 46°50'80" N, 084°22'23" W, 190 m, stems to 0.5 m tall, hips bright red and subglobose to pyriform, 22 Sep 2002, *W.H. Lewis, M. Elvin-Lewis, B. Smith 15801* (MO) (**Fig. 1**). **U.S.A. MISSOURI. St. Louis Co.:** planted in the wild Rose garden with several growing specimens made into vouchers i.e., 16 Jul 2006 *W.H. Lewis* 21175-cult (MO); 22 Apr 2007, *W.H. Lewis* 21176-cult (MO); 30 Jul 2007, *W.H. Lewis 21177-cult* (MO). As of 2017 its rhizomatous population still thrives. Also, still growing is another hexaploid specimen of *Rosa acicularis* ssp. *sayi*. **CANADA. ALBERTA:** W entrance Elk Island National Park (53.60732° N, -112.8621° E), Beaver Creek Hamlet, 8 Oct 2006, *W.H. Lewis 21404,* root tips harvested for counting on 28 Mar 2012; planted in **U.S.A. MISSOURI. St. Louis Co.:** Lewis Rose garden, *W.H. Lewis 21482-cult* (MO). A specimen sent to David Zlesak, **CANADA. ALBERTA:** Peace River (56°24'24"4° N,-117.2771° E), no date, *Olsen et al.*, also proved to be a hexaploid ($2n = 42$). Another representative of this karyotype was collected in **SASKATCHEWAN:** South Saskatoon, 2.1 mi S on RGER 3054 from Hwy 219 to Beaver Creek Hamlet, near roadside in dense marshy aspen woodland, 51°58'15" N, 106°40'14" W, 1591 ft, shrubs with straight multiple stems 0.8 m tall, rhizomatous shoots collected for cultivation, 10 Oct 2006, *W.H. Lewis & M. Elvin-Lewis 21050* (MO). Flow cytometry of leaflet DNA conducted in 2007 indicated it as 6x. **U.S.A. NEW HAMPSHIRE. Carroll Co.:** Moultonborough (43.75676° N, -71.39314° E) below Bald Knob, 29 Sep 2010, *A.B. Schori s.n.* (MO, NH, NHNHB). This specimen from one of the most southerly distributions of *Rosa acicularis* in Eastern North America was established in the Lewis Rose Garden. **U.S.A. MISSOURI. St. Louis Co.:** wild Rose garden, 6 Sep 2002, *W.H. Lewis 21483-cult* (MO). The plant grew well for several years and chromosome counts from its root tips indicated it also was hexaploid, $2n = 42$. The bush, with upright stems up to 45 cm and pink petals, appeared healthy and robust in St. Louis Co., however, the plant suddenly died after 3 years, possibly because of the unusually high heat (temperatures over 100F for several weeks) and drought it experienced in its final growth year. While essentially growing in the same shaded environment as the specimens from Lake Superior and A berta, and in spite of some watering during that period, it was obvious that this unique hexaploid, which had adapted to growing in a cool environment, was less tolerant of this uncommon environmental event. It is noteworthy that a similar response to transplantation of another hexaploid species, *R. nutkana* from the cool wet region of Washington State to the dry heat of southern California was reported by Erlanson (1934) in that new growth was stunted and shriveled and flowering was much delayed (Erlanson 1938). Similarly delayed flowering has frequently been observed when stone fruit cultivars adapted to northern climates are grown in more southern and mild water winter climates. This phenomenon is considered to be due to the accumulation of insufficient chilling temperatures (David Byrne, pers. comm., 2017).

The first recognition of the possible existence of tetraploids ($2n = 28$) in North America was reported in Texas by Florey (1940). This specimen growing at the Texas Agricultural Experiment Station was cited as *Rosa acicularis* var. *engelmannii* (now in synonymy). Because of the oddity at this time and the lack of information regarding its origin this ploidy number was looked upon with some doubt and generally discounted. However, additional data on other North American specimens of *Rosa acicularis* growing there and possibly collected in Alaska and other Northern states did indicate that diploids, tetraploids and hexaploids can tolerate this humid subtropical climate (Florey 1950). Recent collections by Paul Olsen, frequently in collaboration with Margit Schowalter and/or B. Bennett on the Canadian prairies and the Yukon Territories, respectively attest to its occurrence there. From Paul Olsen's viewpoint he considers this ploidy number to be more common than the hexaploids on the prairies an observation that we agree to. These collections are as follows:

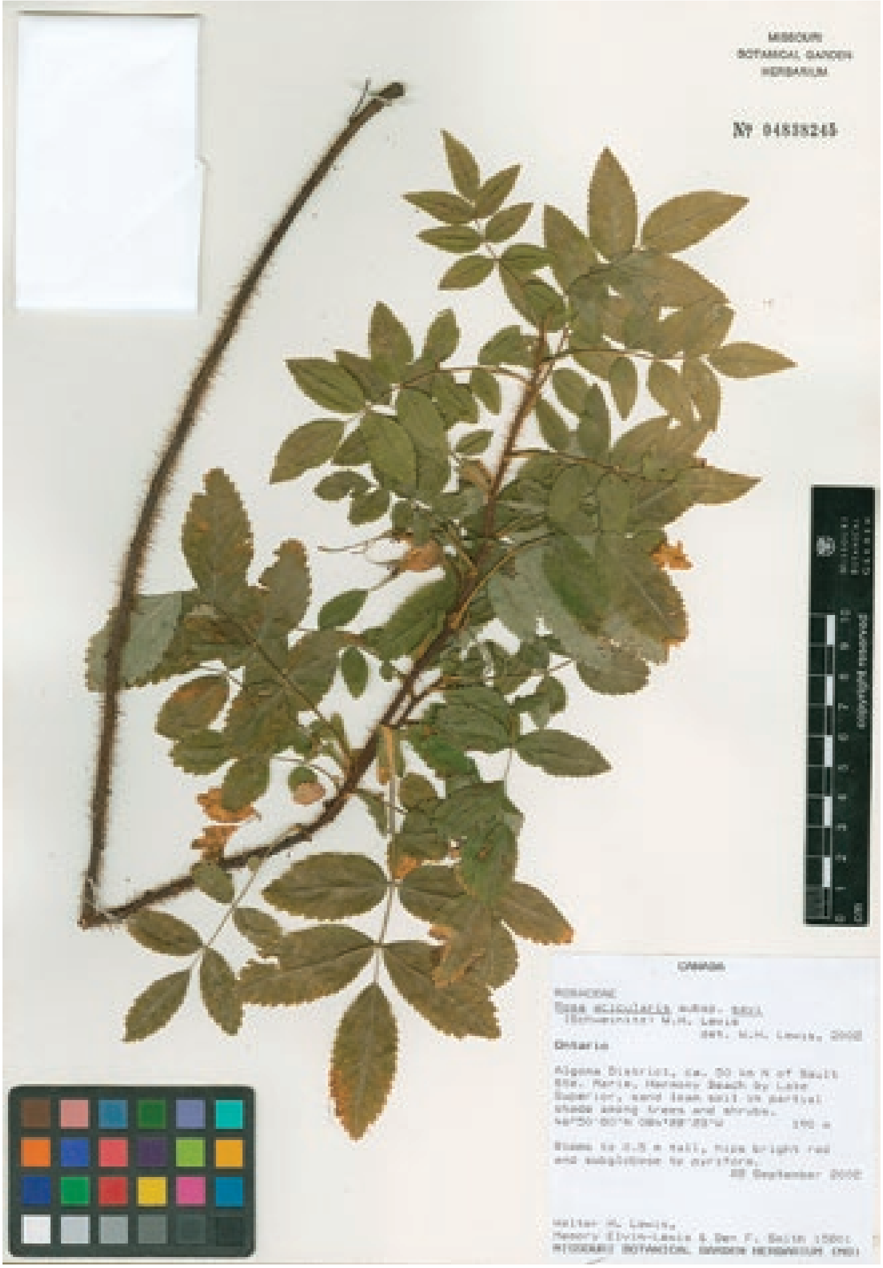

Fig. 1. *Rosa acicularis* Lindl. subsp. *sayi* (Schewin.) W.H. Lewis, $2n = 42$.

CANADA. ALBERTA: Woodbend (53°41'7" N, 113°68'5" W), 2247 ft), 2007, *P. Olsen s.n.,* was received 5 Nov 2010. Its root tip chromosomes were $2n = 28$. It was added to the Lewis Wild Rose garden in St. Louis, Missouri and a voucher made on 10 Nov 2010, *W.H. Lewis 21401-cult* (MO). Another specimen provided to David Zlesak from **CANADA. ALBERTA.** Edmonton, (53°54'49"3° N, -113°50'49" E) *Paul Olsen s.n.*, *no date,* was also $2n = 28$. This city has a prairie-steppe type climate experiencing mild summer (60–70°F) weather and winters reaching 0°F with snow cover. **MANITOBA:** 17 mi E of Winnipeg on the Trans Canada highway, at the clover-leaf with the jct. of Highway 12 (49°42'115" N, 96°39'09.03" W), growing in a fairly wide ditch on prairieland, referred to as the 'George P' rose, April 2000, *Paul Olsen s.n.* (pers. comm., Paul Olsen & Margit Schowalter 2016). According to the Köppen-Geiger classification, nearby Winnipeg is characterized by a humid continental, subtype continental climate with strong seasonality, severe cold (12° F to -6° F winters), no dry seasons and warm summers in the mid-70s°F and 2192 hours of sunshine per year (Climatemps.com). It was received as a potted rooted cutting on 5 Nov 2011 and placed under mist in the Washington University greenhouse as *W.H. Lewis 21399-cult* (MO), its root tip chromosomes were established as a tetraploid (2*n* = 28) and then planted in the **U.S.A. MISSOURI. St. Louis Co.:** Lewis wild Rose garden with a voucher made 23 Jun 2012, *W.H. Lewis 21471-cult* (MO). The specimen still thrives in 2016. **CANADA. YUKON TERRITORY:** Yukon, Whitehorse, Cowley Creek, common in *Picea glauca, Populus tremuloides, Pinus contorta* forest, growing with *Arctostphylos uva-ursi* and *Solidiago simplex*, 60°35'24" N, 134°53'48" W, 712 m, 01 Aug 2010, *B.A. Bennett & R. Mulder 10-412* (YG). This city can average -1°C (30.2°F) with very cold temperatures during January as low as 14°C (57.2°F) and 14°C (57.2°F) in July (climatemps.com). Living specimen received on 10 Aug 2010 and planted in the **U.S.A. MISSOURI. St. Louis Co.:** Lewis Rose garden, a number of vouchers were collected in subsequent years, stems upright, to 25 cm tall, petals pink, 28 Mar 2012, *W.H. Lewis 21482-cult* (MO) (**Fig. 2**); stems branching 1 m tall, 21 May 2013, *W.H. Lewis 21507-cult* (MO); its root tips counted 13 Mar 2012, 2*n* = 28; **YUKON TERRITORY:** Whitehorse, common in a meadow with *Fragaria* (60°45'07" N, 136°02'35" W, 2172 ft), no date, *B. Bennett s.n.*; *R. acicularis* collected in the town of Whitehorse, from the wild part of his backyard of Bruce Bennett 60°59'07" N, 134°89'556" W, 726, no date, *B. Bennett*. **U.S.A. MISSOURI:** Rose garden, residence of Walter Lewis and vouchers made on 4 Apr 2010, *W.H. Lewis 21405-cult* (MO); 21 May 2013, *W.H. Lewis 21505-cult* (MO). Root tip chromosomes were counted on 13 Feb 2011 2*n* = 28. For 5.5 years this specimen is still growing in the Lewis Rose garden.

An early report by Florey (1950) indicated that *Rosa acicularis* var. *engelmannii* (in synonomy) known in the North West United States and growing at the Texas Agriculture Experiment Station, College Station was a diploid.

Several diploid specimens of *Rosa acicularis* Lindl. ssp. *sayi* (2*n* = 14) have also recently been identified in Alaska, Alberta and Manitoba. **U.S.A. ALASKA:** Wasilla, growing wild with deep red flowers on a 5 acre plot at his home on Douglas Drive (61°45'31.22" N, 150°02'30.38" W), 26 Apr 2009, *Kevin Irvine s.n.* and sent to Paul Olsen for forwarding to David Zlesak for karyotyping of its root tips (2*n* = 14, 2x). A potted specimen labeled "Willy" was received from David Zlesak on 5 Nov 2010 and placed in the Washington University Greenhouse where a voucher was made on 10 Nov 2010, *Walter H. Lewis 21400-cult* (MO) (**Fig. 3**) before it was placed in the **U.S.A. MISSOURI. St. Louis Co.:** Lewis Wild Rose Garden, its first 3 flowers collected 27 Mar 2011, had very small petals with a stamen count of 27; it was still living in the spring of 2017, but died during the summer. **CANADA. ALBERTA:** Sandy Beach Summer Village, shore line of W side of lake, edge of scrub with aspen and grasses, shrub 1.2 m tall; hips red, globose to oburceolate and varying in size, 50°47'41" N, 114°02'28" W, 2820 ft, 07 Oct 2006, *W.H. Lewis & M. Elvin-Lewis 21042* (MO). Its DNA diploid status was determined by flow cytometry in 2007. **MANITOBA:** near Minnedosa, E crossroad 262 jct. and Hwy 16, small lake edge, 0.2 N on gravel road by deciduous forest with *Typha* and grasses on verge, shrubs with upright stems, 1 m tall; hips red, urceolate to globose, one complete stem cut in 3 pieces, 50°13'41" N, 099°45'08" W, 1711 ft, 06 Oct 2006, *W.H. Lewis & M. Elvin-Lewis 21037* (MO). **U.S.A. MISSOURI:** wild Rose garden, 08 May 2014, *W.H. Lewis 21520-cult* (MO). Chromosome count 2*n* = 14.

According to Kroon and Zeilinga (1974), hybridization between normal diploid, tetraploid and hexaploid species in *Rosa canina* L. could affect the polyploid status of their progeny. Reference to Shinwari and Shinwari (2003), indicates that numerous rose species growing in Eurasia in sects. *Pimpinellifolieae, Cinnamomeae,* and *Caninae* are also known to possess polyploid series with a basic number of 7. In order to stabilize hybrids between distantly related *Rosa* species, it is not unusual to discover that hybridity is accompanied by polyploidy such as was evident with a specific series of pentaploid rose species (Ritz et al. 2011). For example, *Rosa canina* L. a stable sexual pentaploid has achieved permanent odd polyploidy through complex evolutionary mechanisms (Lim et al. 2005). The nature of polyploidy as related to the evolution of taxa has been an area of interest and controversy for over a half a century and the application of various molecular tools to resolve these issues are now being employed (Kaarthik 2004). For example, a haplotype network (statisical parsimony) of the glyceride 3-phosphate dehydrogenase (GADPDH) nuclear gene was utilized to decipher the evolutionary history of Eastern polyploid species of the *Rosa carolina* complex (sect. *Cinnamomeae*). This geneological approach indicated that only Eastern diploids were involved in the origins of the three polyploid species (Joly et al. 2006). Phylogenetic relationships determined by chloroplast DNA sequences suggest that hexaploid and octoploid populations of *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis) may have been derived from multiple independent origins with different maternal parents (Bruneau et al. 2007). The same phenomenon has been reported in many other polyploid species (Doyle et al. 1990; Soltis et al. 1995). It is also possible the higher ploidy levels of *Rosa acicularis* developed via interspecific hybridity, however molecular data to support this hypothesis has yet to be generated.

While the actual ploidy number of *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis as a parent remains unknown, several hybrids (nothospecies) with other North American wild roses have been identified and named. Crosses with diploid species include × *Rosa blanda* Ait. =*Rosa* × *per-axeliana* W.H. Lewis "Per Axel's

Fig. 2. *Rosa acicularis* Lindl. subsp. *sayi* (Schewin.) W.H. Lewis. $2n = 28$.

Rose” and × *Rosa woodsii* Lindl. ssp. *woodsii* Lindl.= *Rosa* × *canadensis*. W.H. Lewis “The Canadian Rose”; with the tetraploid × *Rosa arkansana* Porter = *Rosa* × *harmsiana* “Harms Rose” and with hexaploid species such as × *R. nutkana* C. Presl subsp. *nutkana* = *Rosa* × *engelmanii* (S. Watson) subsp. *occidentalis* W.H. Lewis (nothotaxon nov.) nov. hybr. “Western Rose”; *R. nutkana* C. Presl subsp. *macdougalii* (Holtz) Piper = *Rosa* × *engelmannii* (S. Watson) Erlanson subsp. *britannicae-columbiae* W.H. Lewis “British Columbia Rose”; *R. nutkana* C. Presl

Fig. 3. *Rosa acicularis* Lindl. subsp. *sayi* (Schewin.) W.H. Lewis. 2*n* = 14.

subsp. *engelmannii* = *Rosa* × *engelmanii* subsp. *engelmannii* W.H. Lewis "Engelmann's Rose" (Lewis 2016). A variety of online sources are also available which shows where other Rosa species can be found in North America where *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis is also known to grow e.g., Plants data base (www.usda.gov) and BONAP natural plant atlas, which shows county U.S.A. distributions (www.bonap.org) are noteworthy. These maps are useful in understanding how many nothospecies evolved (Lewis 2016).

PLOIDY DISTRIBUTIONS IN EURASIA

According to Popek et al. (1991) variations in chromosome numbers of *Rosa acicularis* in Eurasia appear to be dependent upon the geographic latitudes and climate where they might be found. Early studies by Täckholm (1920) who studied specimens growing in botanical gardens in Kew Garden (United Kingdom), Hortus Bergianus, Stockholm and Uppsala (Sweden), Copenhagen (Denmark), and Oslo (Norway) indicated that $2n = 28$ and $2n = 48$ were also represented in these collections along with the predominant chromosome specimens of $2n = 56$, although their exact origins are unknown. Hara (1952) also described an octoploid native to Honshu Island, Japan.

Studies in Russia attest to the widespread presence of this species as an octoploid. For example, this ploidy number ($2n = 56$) has been found throughout Siberia and Altay Mountains (45°0' E,99° N) in the extreme east of Kazakhstan (Rostovzeva & Ligus 1978), Krasnoyarsk (56°1'0" N, 93°4'0" E) on the Yenisei River (Muratova et al. 1998), southern Siberia (Krasnoborov et al. 1980), the western Siberia hemiboreal forests "Nauka" of the palearctic ecoregion (Krogulevich 1976, Krogulevich 1984) the southern Tuninkinsk Alps (East Syans) and Sayan Mountains (Krogulevich 1976) the Asia-Pacific region including the Far East (Krasnoborov et al. 1980), the south Far East (Gurzenkov 1973) and the West Chukotka, Anadyr region (Zhukova & Petrovski 1975). Anadyr, the Eastern most town in Russia, has a subarctic climate with long cold winters and cool, short summers and is found at the same latitude of about 64° N as Fairbanks, Alaska (wikipedia.org/wiki/Anadyr_(town)(64°44' N, 177°31' E). Also, recent studies in the Republic of Sakha (Jakutein), known for its extreme hyper continental climate, indicate that octoploids ($2n = 56$) there are most probably found in the vast forest taiga region which covers 47% of the region with larch trees dominating in the north and fir and pine in the south (Popek et al. 1991).

Hexaploids ($2n = 42$) have been discovered in several climatic zones. These are also known in Russian Siberia in Krasnoyarsk (56°1'0" N, 93°4'0" E) on the Yenisei River where its continental climate is characterized by long and very cold winters and short but warm summers (Muratova et al. 1998). Also in the south Siberia Region in Yakutsk (62°02' N,129°44' E) extreme temperature ranges are characteristic in this humid subarctic continental climate with temperatures reaching -40°F/C in the winter and summer temperatures ranging from 68°F up to +38.4°C (101.1°F) (Rostovzeva 1977). They have also been located in the northern region of the Republic of Kazakhstan within the range of 50 ± North latitude which borders the Russian Ural and Siberian districts with its steppe and taiga (Popek et al. 1991). Similarly, they are present in Turukhansk (65°49'0" N, 87° 59 0" E) within the north taiga with its continental sub-arctic climate eliciting mild summers and severely cold winters and the Russian Far East also known for its dry sunny winter weather where northern and southern climate zones overlap in the temperate mixed rain forests of Ussuri and South Sakhalin-Kurulin (Muratova 1998; Volkova & Melnikova 2001; Sedeinikova 2002). Within this region, this karyotype has also been discovered in Primorsky Kray, valley of the river Kazachka (Volkova & Mel'nikova 2001).

Chromosome studies on *Rosa acicularis* seedlings propagated from various parts of Russia by Sedelnikova and Pimenov (2002) indicated that proportional differences in karyotypes exist in the north and south taiga (boreal forest) zones (Turukhansk) and Priangarie. (Irkutsk Oblast which is also known as Pribaikalie or Priangarie is a federal subject of the Russian Federation, located in the Southern part of Siberian Federal District near Lake Baikal.) Karotypes found under extreme environmental conditions of the north-taiga zone were dominated by octoploids (72%), with fewer hexaploids (28%) found. However, in the south taiga the frequency of hexaploids dominated (60%), and the incidence of octoploids was proportionally lower (30%) with heptaploids at 10%.(Since the same data and reference found in Sedelnikova and Pimenov (2002) is also cited in (Sedelnikova 2002) found in the IPCN Chromosome reports (www.tropicos.org/Project/IPCN) the latter is likely an error.)

Heptaploids are also known to Krasnoyarsk (56°1'0" N, 93°4'0" E) in Russian Siberia (Muratova et al. 1998). However, it is unclear as to the status of the sterility of these heptaploids found there and elsewhere in Russia and such studies are warranted. This is because a high percentage of abortive pollen (65%) was found in one specimen from Golovin, Alaska (64°32'41" N, 163°1'39" W), which may also have been a heptaploid ($2n = 49$). Because of its locality, it is possible it was a cross between an octoploid Eurasian subspecies with a hexaploid of North American origin thus the abnormal pollen was a result of irregular meiosis. This phenomenon was affirmed by Eileen Erlanson who produced an artificial heptaploid (*Lewis 2527*) by crossing the 2 subspecies, but unfortunately neither pollen studies nor observations on meiosis were made (Lewis 1959). The current discovery of natural heptaploids growing within the juncture of where octoploids and hexaploids exist in Eurasia is therefore significant.

Moreover, pollen grain measurements made by Lewis (1959) on a variety of specimens considered to represent octoploid and hexaploid *Rosa acicularis* specimens in Eurasia and North America, respectively were generally consistent with the ploidy level of the specimen. For example, among specimens from Eurasia (Outer Mongolia, Sweden and the former USSR) where octoploids predominated, the pollen diameters were between 40.2 µm to 41.5 µm, and in North America where hexaploids were found their diameters were somewhat smaller 37–39 µm. Therefore is it worth noting that a specimen examined from Yakutsk (Kisilyach) 56°1'0" N, 93°4'0" E averaging 39.8 µm made at this early period proved to be consistent with what might be expected for a hexaploid recently identified in this same locality (Rostovzeva 1977). Similarly, from the same latitude, a specimen from Tomsk (56°30'0" N, 84°58'0" E) measured 37.9 µm (Lewis 1959) suggesting it was also a hexaploid. It is noteworthy that studies by Erlanson (1931) of 23 hexaploid specimens of *R. acicularis* indicated that percent mean pollen sterility was low (7.5%) when compared to most diploid and tetraploid North American *Rosa* species thus suggesting the potential viability of this karyotype.

Ethnobotany of *Rosa acicularis* Lindl.

In North America indigenous populations use *Rosa acicularis* Lindl. for both culinary and medicinal uses. For example, in Alaska both native and Eskimo populations employ its seeds (hips) for ice cream or freeze them for future use (Jones 1983), as well as making them into jams, jellies, marmalade, ketchup and syrup, and as a rich source of Vitamin C (Heller 1953). Also, beekeepers value its flowers as the source of nectar for their honeybees (Peterson 1989).

Similarly, fruit jellies are made by Thompson First Nation (Turner et al. 1990). Fruit is eaten raw by the Koyukon (Nelson 1983) Cree (Leighton 1985), Blackfoot (McClintock 1909), Montana (Blankinship 1905) and Tanana (Guedon 1974) who also cooked it with grease and sugar (Kari 1985) whereas its rind is preferred by the Okanagan-Colville (Turner et al. 1980). Also, raw petals are eaten by the Tanana (Kari 1985), buds by the Ojibwa (Reagan 1928) and peeled young tender shoots by the Thompson (Turner et al. 1990). Teas are made by the Thompson from its hips and shoots and during pit cooking these plant parts are also placed over the food to add flavor and prevent burning (Turner et al. 1990). Also, numerous studies reviewed by Crane (1990) indicate that a wide variety of wildlife such as rabbits, bears, beavers, deer, elk, mountain goats, pronghorn, and moose depend upon *Rosa acicularis* Lindl. for sustenance by eating its hips, stems and foliage.

Aboriginal North Americans utilized this species for a wide variety of medicinal applications. For example, the Tanana make use of decoctions of stems and branches for “weak blood,” colds, fevers, stomach troubles, diarrhea and a bark infusion as an emetic (Kari 1985). Chewed leaves are applied to bee stings among the Okanagan-Colville (Turner et al. 1980). The Iroquois use leaf and bark infusions as eye drops for blindness (Herrick 1977) and the Cree (Leighton 1985) use root infusions to treat sore eyes. The Iroquois drink twig-bark infusions for a difficult birth (Herrick 1977) whereas the Thompson chew the hips to hasten labor and drink root decoctions after birth and to treat syphilis (Turner et al. 1990). Root decoctions are also taken by the Cree to treat cough (Leighton 1985). Among the Blackfoot its root, prepared as a drink, is used to treat children with diarrhea.

The Okanagan-Colville (Turner et al. 1980) has several uses of branch decoctions for washes so as to remove the human scent from hunters or to permeate fishing nets with good luck. Also, leaf and branch decoctions are employed in sweat baths to wash the body and hair. Teas made from its branches are also said to protect drinkers from bad spirits and ghosts and when used as a wash to remove jinxes. To prevent spirits of the deceased from returning branches are placed around a house (Turner et al. 1980).

Cradle hoops are made from splitting the wood by the Thompson (Turner et al. 1990), and hips are strung for jewelry or hollowed to make a toy pipe by the Cree (Leighton 1985). Its petals were made into perfume in Montana (Hart 1976).

According to the Great Soviet Encyclopedia (1970–1979), fruit (hips) of a number of wild roses including (*Rosa acicularis* Lindl. subsp. *acicularis*) are available fresh, dried or canned as a rich source of Vitamin C. To treat vitamin deficiencies the fruit pulp is ingested in the form of tinctures, syrups, candies, or lozenges. Also to heal several varieties of dermatological and mucous membrane conditions as well as to treat trophic ulcers topical applications of its oil or oil extracts derived from rose petals are applied topically. Tanning agents and dyes are also made from the fruit and roots of some *Rosa* species (Saakov & Rieksta 1973). While most of these examples are possibly commercially derived it is evident from parallel choices known to North America that some of these applications are based on traditional knowledge.

Horticultural uses

Both in North America and Eurasia *Rosa acicularis* Lindl. can be found in gardens as a flowering shrub and because of its armature may also be preferred for hedges. In North America sale of this species is listed on a number of internet sources, with nurseries promoting it for its rich red color and cold hardiness. In Russia, according to Tatyana Shulkina (Personal communication 2016) it is common knowledge that a number of wild roses including *Rosa acicularis* Lindl. subsp. *acicularis* are used similarly. Within this context when used in landscaping this species is well adapted to pruning (Interactive Agricultural Ecological Atlas of Russia and Neighboring Countries, 2003–2009). However in a survey of the potential of local plants for horticultural purposes, Wei et al. (2015) considers that *Rosa acicularis* L. found in the Helan Mountains of Inner Mongolia is only of secondary importance when utilized as an ornamental shrub.

On the other hand without data to attest to their ploidy numbers one can only estimate from local sources what these might be. For those plant breeders looking for genes associated with a specific blooming time, richness of color, cold hardiness, or some other salient feature the outcome is more likely to be predictable if the specific karyotype of the *Rosa acicularis* Lindl. is known. For example, horticulturists selecting for a specific blooming time would have to know that earlier blooming times are consistent with higher ploidy levels as is the likelihood of identifying cold hardiness. However care must be taken to appreciate that other genes associated with the viability of the species under its natural environment may not allow certain of these hybrids to grow elsewhere where conditions are different. For example experimental studies of Erlanson (1934) and Erlanson (1938) showed that octoploids when transplanted from northern latitudes were less likely to tolerate climatic zones of Michigan where early spring warmth can promote a flush of growth which is susceptible to killing frosts that sometimes follow. She concluded this lack of tolerance in these earlier flowering, slower growing roses was related to their structure in possessing “shorter flowering laterals and a one- to few-flowered inflorescence.” She also recognized that certain physiological features that have evolved, particularly in octoploids, are directly related to their survival in arctic or boreal habitats. She further reiterated that this karyotype from northern latitudes responds to slight increases in temperature known there in early spring in putting out foliage and flower buds which continue to develop under the slow incremental rise in temperatures throughout the very short growing season. However, when transplanted to lower latitudes such as Michigan or Missouri where alternating spring thaws and late freezes occur, they are unable to prevent serious damage to their new growth. This phenomenon has also been observed among stone fruit such as apricots and plums adapted to northern climates. When these are grown in mid latitude regions they tend to begin breaking early due to their requirement for moderate chilling and low heat (David Byrne, pers. comm., 2017).

A number of horticulturists have attempted to develop artificial octoploids with the purpose of producing needed hardiness; however their ability to tolerate the St. Louis climate is indicative of their fragility at this latitude. For example, Robert Erskine in Alberta crossed two wild *Rosa acicularis* Lindl. specimens called ‘Aurora’ ($2n = 28$) and ‘Leafland Glow’, resulting in an octoploid ($2n = 56$) called ‘Kinistino. It was received as a rhizomatous shoot 5 Nov 2010, placed under mist at the W.U. Greenhouse, and planted in the wild Rose garden. The next year its stems were 0.3–0.4 m tall, and upright, with previous year pedicels curved with elongated hips (*W.H. Lewis, 21397-cult* (MO). It died early in 2011. Another specimen representing a cross between ‘Kinistino’ and Dr. Buck’s thornless *Rosa multifora* cross of IT9 and ITI8 which was grafted on *Rosa multiflora* root stock was received 5 Nov 2010 from P. Olsen via D. Zlesak, and was placed in the wild Rose garden (*W.H. Lewis 21398* *cult*) where it was still living in 2016. The reason for this is possibly related to the tolerance of *Rosa multiflora* to the Midwestern climate. Also sent from P. Olsen and M. Schowalter via D. Zlesak on 5 Nov 2010 was another specimen representing a cross between 2 other *R. acicularis*. This came from the University of Alberta's Devonian Botanical Garden in Devon, Alberta which is Canada's most northerly botanic garden (53°24'27.27" N, 113°45'34.94" W). This rhizomatous shoot was propagated under mist at the Washington University greenhouses and placed in the wild Rose garden as *W.H. Lewis 21397-cult* (MO) and is a tetraploid (*2n* = 28). The next year it produced upright stems, 0.3–0.4 m tall, with its previous year's pedicels curved with elongated hips. Unfortunately it did not survive during that year's growing season. Another referred to as "George Pegg" collected at the Georges Bugnet plantation (53°52'14.25" N, 114°22'58.13" W, 2263 ft, 20 Oct 2011 *M. Schowalter s.n.* is *2n* = 28.

Chromosome data do not often exist for known horticultural hybrids derived from crosses of North American *Rosa acicularis* Lindl. ssp. *sayi* (Schwien.) W.H. Lewis and horticultural varieties. From consulting with David Zlesak, Paul Olsen, and Margit Schowalter regarding the parentage of some of these it is apparent that the precise parentage of which native wild roses were used remains questionable. For example, Paul Olsen suggests that the wild rose parentage of 'Georges Bugnet' and 'Therese Bugnet' is more likely to be *R. woodsii*. The basis for this conclusion is the fact that 'Therese Bugnet' is a diploid like *R. woodsii* rather than *Rosa acicularis* which would more likely be a hexaploid in this region and that *Rosa woodsii* is much more common than *Rosa acicularis* in the area where he lived (northwest of Edmonton, Alberta). Robert Erskine's 'Caroyal' ('Hansa' × 'Lac La Nonne') is infertile both ways and therefore perhaps a triploid, which if true would prove that 'Lac La Nonne' is a tetraploid. He believes 'Georges Bugnet' could have been wrong with the parentage he gave for 'Lac La Nonne' since he may not have been able to differentiate between *Rosa acicularis* Lindl. and *Rosa woodsii* Lindl. He was likely wrong in the parentage he gave for 'Therese Bugnet' and that another species would probably have been the native species he used in his breeding programs.

Under the circumstances it is prudent to eliminate discussions regarding 'Lac la Nonne', Pelouses the mother plant of 'Therese Bugnet', 'Fanny Heath', 'Caroyal' and 'Julia Bugnet' until further genetic analysis is conducted. Also, early hybridizers and botanists may have confused the identity of certain wild roses; primarily because they often grew together and to some looked similar in that *Rosa woodsii, Rosa blanda* and, *Rosa arkansana* can resemble *Rosa acicularis* by one or more features (W.H. Lewis 1957). During this period some collectors apparently had difficulty in understanding the lack of prickles as a predominant feature of *Rosa blanda* and thus would refer to these specimens as *Rosa acicularis*. Also, current data suggests that others may have inadvertently selected wild rose hybrids including those with *R. acicularis* as one parent (Lewis 2016) or misidentified them altogether (personal communication Paul Olsen (2016). Olsen (2010) has also suggested that the tetraploid *Rosa acicularis* might be a preferable parent when crossing with other tetraploid wild roses such as *Rosa virginiana*.

With these cautionary notes in mind, several varieties claiming *R. acicularis* as one parent have been developed and can be found in "Helpmefind.com roses" (http://www.helpmefind.com/rose/). The earliest was in the United Kingdom when Gunter in 1940 crossed *Rosa acicularis* Lindl. with the hybrid tea rose 'Hollywood' developed by Scintine in 1930 to produce the hardy USDA zone 6b–9b, 'Pikes Peak'. It was introduced into the U.S.A. by the Bobbink & Atkins Company, East Rutherford, New Jersey. The "Shellbrook Rose," now believed extinct, was discovered by Albert J. Porter. This hybrid, with *R. acicularis* was dark red. Another possibly extinct hybrid of *R. acicularis* Lindl. crossed with *R. spinosissima* var. *aitaica* (Willd.) Rehder is called "Altalaris." It was bred by Frank Leith Skinner in 1941 in Canada and bloomed only once in the spring or summer with characteristic white pink edges. Being cold tolerant it grew from USDA zone 2b or warmer. He also crossed *R. acicularis* and *R. rugosa*, which he called "Fanny Heath" that appeared in the 1942 Dropmore Catalogue. This hybrid with fragrant deep red flowers has a cold hardiness to USDA zone 2b "(with light protection) and warmer." Several unnamed *Rosa rugosa* × *Rosa acicularis* hybrids also exist. Also from Canada, a hybrid between *R. acicularis* and an unknown *Rosa* was discovered in 1966 by Walter Schowalter who called it "Halkirk." He described it as being 15 feet tall with small cupped deep rose blossoms. Also in 2003, another Canadian hybridizer, Robert McKay Erskine crossed ‘Harison’s Yellow’ with *Rosa acicularis* and called this semi-double to double form, with medium pink petals, “Haris” rose. However, according to Margit Schowalter (pers. comm. 2016), this heritage remains uncertain.

Also a number of *Rosa acicularis* Lindl. specimens has been made with other species known elsewhere. For example, in Belgium, Louis Lens (sometime before 2001) crossed this species with *Rosa moyesii* Hemsl. & E.H. Wilson. It is hardy between USDA zone 6b through 9b (default). Another unnamed hybrid with similar hardiness has been made with *Rosa majalis* Herman. A very hardy hybrid (USDA zone 3a to 9b) made with *Rosa acicularis* Lindl. subsp. *acicularis* and an unknown parent is also cited as are others with only *Rosa acicularis*.

DISCUSSION

Current data on the distribution of karyotypes of *Rosa acicularis* L. existing on both continents suggests that there is a greater diversity of chromosome numbers than heretofore appreciated. Past generalizations which suggested that higher ploidy levels, such as octoploids found primarily in upper northern latitudes have a survival advantage in severe colder climates than those of less ploidy levels (hexaploids and tetraploids) which tend to predominate in lower latitudes requires closer scrutiny. It is apparent from both the data presented in North America (Table 1) and in Eurasia (Table 2), and illustrated in Figure 4 that latitude is not the only parameter that must be considered since it is evident that both the microclimate and the ecology of a particular region are major selective features in dictating the types of karyotypes which can be present. For example, *Rosa acicularis* plants found in lower latitudes and growing in higher altitudes are more likely to have higher chromosome numbers similar to those growing in higher latitudes. Recently these factors were exemplified by the studies of Sedelnikova and Pimenov (2002), which indicated how variations of karyotypes existed where both climate and latitude differed in the southern and northern regions of Russia. For example, the existence of octoploids (30%) in lower latitudes is likely associated with collections made from boreal forests in high mountain ranges found in these regions. This proportion differs markedly from that of northern taiga collections where extreme environmental conditions occur and octoploids predominate (72%). It is noteworthy that were where hexaploids predominated in the south (60%) a small proportion of heptaploids (10%) were also found. *Rosa acicularis* L. is not alone in responding to environmental influences since these authors also noted that genomic mutations and chromosome abnormalities also seen among other woody plants growing in these regions such as *Betula* L , *Picea* A. Dietr., *Pinus* L. *Quercus* L , and *Ulmus* L. The exception to this rule is the discovery of a diploid in Alaska where hexaploids and octoploids predominate. This unique specimen appears to be able to survive both at USDA hardiness zone 4b of Wasilla, Alaska, 61°45'31.22" N, 150°02'30.38" W and until the summer of 2017 6b in St. Louis (38°37'11" N, 90°19'43" W, 533 ft). The recent discovery of this specimen and others found in Canada at lower latitudes c.a 50° N where it has also maintained a small stature could be the source of discovering the origins of this species as was suggested some time ago by Joly et al. (2006).

Natural heptaploids which occur in Russia wherever octoploids and hexaploids are found (Sedelnikova and Pimenov, 2002) confirms that such crosses are possible. However, their occurrence in lower frequencies than their parents attests to the earlier observations on the aberrant pollen morphology of an artificial heptaploid (Lewis 1959), and as such issues of sterility may exist. Also the rarity of natural occurring heptaploids in North America may be associated with the infrequency of known octoploids. However, this paucity of octoploid collections may be due to where these were made decades ago. It is possible that additional regions in Alaska and adjacent Canadian territories that now are routinely reached by airplane could provide exciting collection opportunities. Not only would such collections serve to extend the range of octoploids, but by doing so could identify heptaploids where there is an interface with hexaploids.

While karyotype data obtained from *Rosa acicularis* growing in botanic gardens in Scandinavia the United Kingdom (Täckholm 1920) and Hungary (Popek et al. 1991), and the Texas Agricultural Experimental Research Station in Texas (Florey 1940; Florey 1950) have some value it is difficult to ascertain the particular range of ecological plasticity of these collections without accompanying data to indicate where the plants originated. In the experimental plot of the senior author in St. Louis, years of observation indicated that some

TABLE 1. North American karyotype distributions (*Rosa acicularis*) according to USDA hardiness zones, Köppen-Geiger classification and geolocations.

Hardiness Zone	Biome*	Provence/State	Locality	Geolocation	Collection Number	2n
1b; Dfc	Cool temperate steppe -45.6°C (-50 °F)	Yukon Territory	Whitehorse	60°35'24" N; -134°53'48" W 60°59'07" N; -134°89'556" W	B.A. Bennett & R. Mulder 10-412 (YG); B.A. Bennet, sn	28 28
2b; Dfc	Boreal moist forest	Alberta	Peace River	56°24'24"4° N, -111°7.2771" E; 89′556" W	P. Olson. sn	42
3a; Dfb*	Extreme humid continental	Manitoba	Minnedosa	50°13'41" N, -99°45'08" W	W H. Lewis & M. Elvin-Lewis 21037 (MO)	14
3a; Dfb*	ibid	Manitoba	Jct. of TransCanada Hwy and Hwy 12, 17 mi E of Winnipeg	49°42'.1155" N, 96°39'09".03 W	P. Olsen, sn	28
3b; Dfb	Temperate/ humid continental climate	Alaska	Golovin	64°32'41" N, 163°1'39" W	Lewis 1959	49; Pollen
3b; Dfb	Truly continental; severe winters, warm summers; no dry season; Boreal moist forest	Alberta	Elk Island National Park	53°60'73"2° N, -112°86'21" E	W.H. Lewis 21404	42
3b; Dfb	Ibid	Alberta	Edmonton	53°54'49"3° N, -113'5049° E	P. Olsen sn.	28
3b; Dfb	Ibid	Alberta	Georges Bugnet Plantation	53°52'14".25 N, 22'58.13" W; Elevation 2263	M. Schowalter sn.	28
3b; Dfb	Ibid	Alberta	Woodbend	53°41'7" N, 113'68".5 W	P. Olsen sn.	28
3b; Dfb		Alberta	University of Alberta Devonian Bot. Garden	53°40'81" N, -113°.75'68" E	P. Olsen, M Schowalter, sn	28
4a; Dfb	Truly continental; severe winters, warm summers, no dry season	Saskatchewan	S. Saskatoon	51°58'15" N, 106°40'14" W	W.H. Lewis and M. Elvin-Lewis 21050 (MO)	42
4a; Dfb	Ibid	Saskatchewan	Sandy Beach Summer Village	50°47'41" N, 114°02'28" W	W.H. Lewis and M. Elvin-Lewis 21042 (MO)	14
4a; Dfb*	Humid continental climate; cold snowy winters and warm summers	Ontario	Sault Ste. Marie, Harmony Beach	46°50'80" N, 084°22'23" W	W.H. Lewis, M. Elvin-Lewis, B. Smith 15801 (MO)	42
4b; Dfc	Continental subarctic; long, severe cold winters & short mild summers	Alaska	Wasilla	61°45'31".22 N, 150°02'30.38" W	Kevin Irvine sn	14
5a; Dfb	Subarctic	New Hampshire	Moultonborough	43°75'67"6° N, -71°39'31"4° E	A. B. Schori sn. (MO, NH, NHNHB)	42
6a, 6b; Cfa	Temperate hot summers, no dry season; "subarctic" at mtn. base	West Virginia	Ice Mountain	c.a. 39°21'48" N, 78°28'01" W	W.H. Lewis 2509 (MO)	42
6a; Cfa	Humid subtropical	Missouri	Richmond Heights, St. Louis *	38°37'11" N, 90°19'43" W, 533 ft	W.H. Lewis	14, 28, 42
8b; Cfa	Humid, subtropical; no dry season	Texas (collected elsewhere, in exp. garden)	Texas Agric. Exp. Stat. College Station **	30°36'54.0396" N, 96°20'32.9136" W	W.S. Flory s.n.	14, 42, 28,

For specific temperature ranges refer to climatemps.com or weatherbase.com
*Transplanted specimens, original localities known, experimental plot
**Transplanted specimens, original localities unidentified, experimental plot

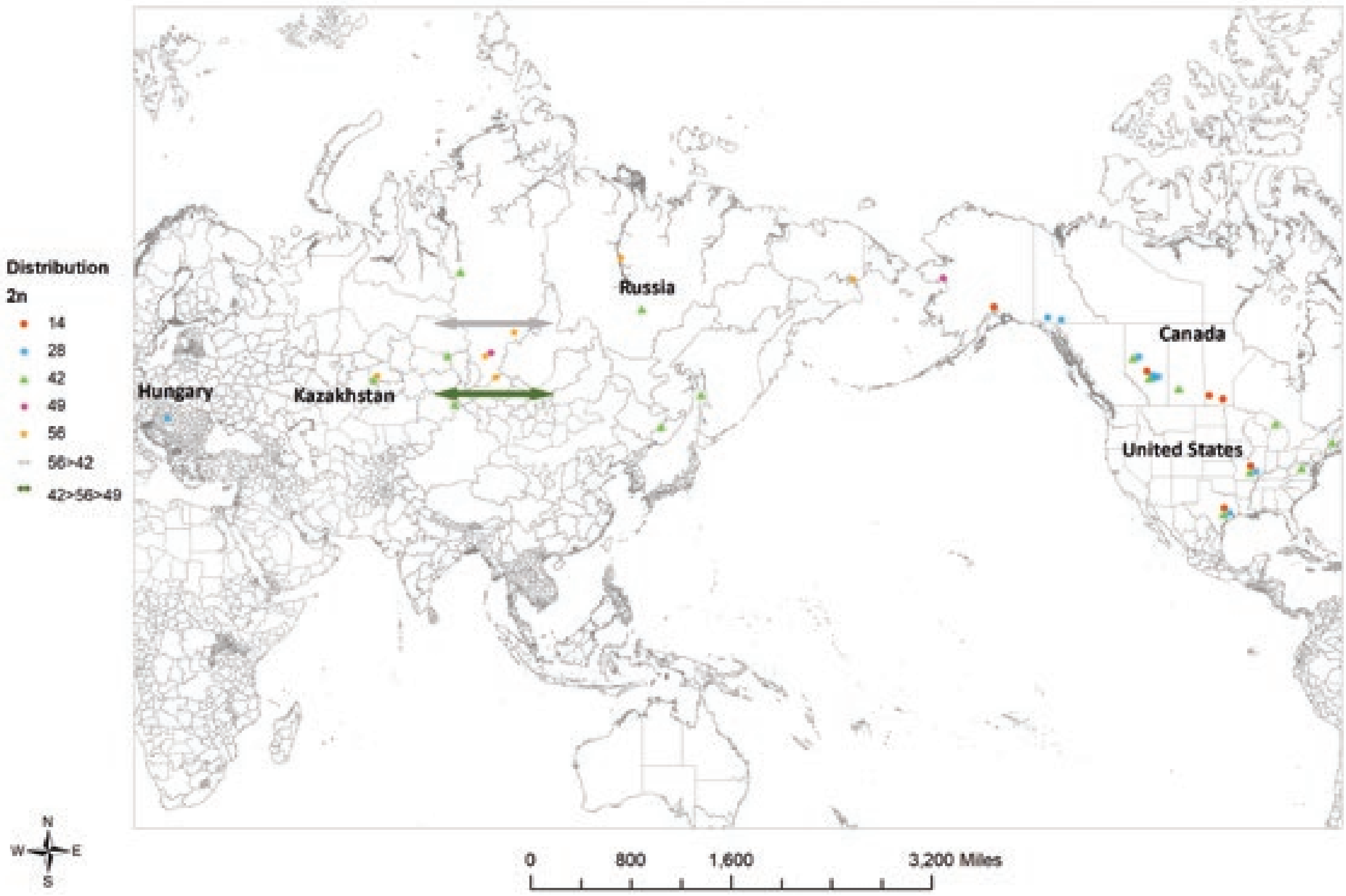

Fig. 4. Karyotype distributions (*Rosa acicularis*) in Eurasia and North America.

Table 2. Eurasian karyotype distributions (*Rosa acicularis*) according to Trewartha and Köppen-Geiger* climate classifications and geolocations.

Classification	Description	Country	Locality	Geolocation	Author	2n
2.3.3; Dfb; Dfc	Temperate climate, overlapping n and s climate zones with tiaga	Russia	Ussuri and South Sakhalin-Kurulin Russian Far East	45°50' N & 54°24' N	Volkova & Melnikova 2001	42
2.4.1	Extreme continental climate	Republic of Kazakhstan		50° N latitude	Popek et al. 1991a	56; 42
2.4.1	Continental	Hungary	Budapest	47°31'2.92" N,19°04' 23.41" E	Popek et al. 1991b	28
2.4.2; ET	Extreme hyper continental, tundra climate	Russia	Republic of Sakha	66°24' N, 129°10' E	Popek et al. 1991a	56
2.4.2; Dfb	Humid continental climate with warm summers, severe winters and no dry season in boreal moist forest biome	Russia	Tomsk	56°30'0" N, 84°58'0" E	Lewis 1959	42; pollen size
2.4.2; Dfb	Ibid	Russia	Krasnoyarsk	56°1'0" N, 93°4'0" E	Muratova et al. 1998	56; 49
2.4.2; Dfb	Ibid	Russia	Siberia, Altay Mtns. extreme E of Khazikstan	45°0' E, 99° N	Rostovzeva & Ligus 1978	42
2.4.3-ECld; Dfd	Continental subarctic climate		Turukhansk	65°49'0" N, 87°590" E	(Muratova 1998; Sedelnikova & Pimenov 2002)	42
2.4.3; Dfc	Subarctic or boreal climates		West Chukotka, Anadyr region	64°44' N, 177°31' E at Anadyr	Krogulevich 1976	56
2.4.3; Dfd	Humid subarctic continental climate		Yakutsk, South Siberia Region	62°02' N,129°44' E	Rostovzeva 1977	42
2.4.3; Dfd,	Extreme subarctic climate		Yakutsk (Kisilyach)	56°1'0" N, 93 4'0" E	Rostovzeva 1977	42
Dwd, Dfd	Subarctic, humid continental or temperate boreal climates		Southern Siberia	49°8'8" N 87°33'46" E / 49.13556° N 87.56278° E / 49.13556; 87.56278	Krasnoborov et al. 1980	56
2.4.3; Dwd	Subarctic or temperate boreal forests	Russia	Sayan Mountains	53°15'4.20" N 94°58'16.79" E	Krogulevich 1976	56
Dfc, Dwc, Dsc, Dfd, Dwd	Moist, subarctic climate, large temperature range, long and cold winters	Russia	North Taiga	Spans Eurasia at 50° N latitude	Sedelnikova & Pimenov (2002)	56>42
Dfb, Dwb with longer summers	humid continental, longer summers in the South	Russia	South Taiga, Turkansk, (Boreal Forest), Priangarie Region	Spans Eurasia at 40° N latitude	Sedelnikova & Pimenov (2002)	42>56; >49

* Refer to Peale et al. 2007 for Updated world map of the Köppen-Geiger climate classification: Map of Russia

collections from northern areas of North America had difficulty surviving the continental climate for extended periods of time and were particularly susceptible to the elevated heat of the summer of 2012 and 2017. Since extreme temperature fluctuations are becoming more common place, future studies associated with environmental stress should consider this aspect when designing long-term experiments

While it is appreciated that polyploidy is associated with genome-wide changes in gene expression and epigenetic modifications, studies on these aspects in *Rosa acicularis* Lindl. and its various karyotypes are only now being explored (Fougère- Danezan et al. 2014; Ritz et al. 2011). One would expect to find a wide range of diversity associated with genotypic and phenotypic expression, cell size, gene dose effect, and various levels of heterozygosity, gene silencing and genetic and epigenetic interactions to be in play (Dewitte et al. 2012). This is evident among some of the karyotypes represented in *Rosa canina* L. which depend upon different gene expressions to insure their survival in specific climatic zones as they increase their geographic range or extend into higher altitudes (Ritz et al. 2011). Unlike other woody plants and trees, where chromosome abnormalities and mitotic irregularities are common in harsher climates, this has not been the case with this species (Sedelnikova & Pimenov 2002). For example octoploids, which live in the cold harsh environments in the north taiga zone (Sedelnikova & Pimenov 2002) or those produced artificially are somehow prevented from adapting to climatic zones where hexaploids, tetraploids and diploids have the advantage. That is not to say that the latter cannot be found in northern latitudes, it is just that their frequency is lower. For example, our observation that grafting of an artificial octoploid onto the hardy *Rosa multiflora* rose stock provided it needed protection to survive the St. Louis Midwestern climate is relevant, since other octoploids did not survive. It is possible that the root system of octoploids are not suitable to lower latitudes and are likely similar to other arctic plants which require shallow root systems so they can avoid the permafrost and grow in an active layer. Similarly, the acicular nature of its stems and buds suggests these were evolved to serve as a layer of protection against cold weather. Also, the adaptation of these octoploids to complete their growing cycle and produce seed sets in the short growing season and long hours of sunlight of the north is clearly a selective advantage to their survival. In addition the larger leaves characteristic of higher ploidy numbers of *Rosa acicularis* Lindl. are likely to have a more efficient photosynthetic process for extreme colder climates than other arctic plants which have evolved smaller leaves to conserve water in the more arid regions of the arctic (Royal Geographical Society (RGS) 2016). These observations could serve as a base line for future research as warmer climates (Rubel & Kottek 2010), which are anticipated to occur during this century and beyond affect the econiches of a variety of plants and animals. Some in the scientific community have predicted that mass extinctions of at least one third of all species may occur by 2050 (Brugger and Rubel, 2013). Because ploidy levels are an adaptive feature of this species any changes in the proportion of existing karyotypes in a specific region are likely to serve as a useful indicator of such environmental changes.

Little is known regarding the compatibility of crosses made between various karyotypes in this species however it is recognized that permanent hybrids are unlikely to occur when intergenomic recombination of allopolyploids takes place. This can lead to genetic segregation of parental characters in the progenies. However, vegetative propagation may overtime lead to sexually stable offspring of these hybrids (David Byrne, personal communication 2017). In other taxa chromosome pairing is disturbed, functional *n* gametes are lacking but first-division restitution (FDR) gametes are exclusively produced (Ramana & Jacobson 2003). What is essential is that recombinant chromosomes exist between the two parents (Gonzalez et al. 2004) as is evident among natural hybrids among North America Rosa (Lewis 2016) and others derived by horticultural crosses made with commercial varieties to affect cold hardiness. Therefore these phenomena are clearly related to the success of hybridizing other rose species with the various karyotypes of *Rosa acicularis* Lindl. and thus knowing the chromosome number and possibly section of each parent is important, if not essential.

The ethnobotany of both subspecies appears to be similar in spite of the fact that data from North America is primarily related to aboriginal uses and that of Russia is possibly derived from traditional knowledge sometime in the past. This is especially true for its use as food, and especially the high regard for its Vitamin C content in its fruit/hips. Since studies into the pharmacological basis of other medicinal uses has yet to be accomplished one can only presume that there are similar bioreactive compounds found among both subspecies and others unique to one or the other. It should be emphasized that those with high ploidy numbers may contain amplified amounts of certain substances. Studies regarding compounds associated with pharmacological effects known among other closely related Rosaceae should provide possible leads whenever this aspect is explored further (Lewis & Elvin-Lewis 2003).

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