Molecular markers indicate that the narrow Québec endemics *Rosa rousseauiorum* and *Rosa williamsii* are synonymous with the widespread *Rosa blanda*

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Abstract: *Rosa rousseauiorum* Boivin and *Rosa williamsii* Fern. are two rare roses in eastern Québec, whose taxonomic status is controversial. Morphological characters alone do not clearly differentiate these two taxa from each other or from the morphologically variable and widespread *Rosa blanda* Ait. We evaluated the taxonomic status of these two taxa, and of two other *R. blanda* segregates, *Rosa subblanda* Rydb. and *Rosa johannensis* Fern., through an analysis of RAPD, ISSR, and AFLP markers. We surveyed 86 individuals from 36 populations in eastern North America. Despite a high degree of polymorphism, principal coordinate analyses and the weighted pair group method with arithmetic averaging suggest no clustering of individuals that correspond to taxonomic boundaries. However, the closely related *Rosa palustris* Marsh. is clearly differentiated from the *R. blanda* s.l. taxa. When populations of *R. blanda* west of Québec are included, the principal coordinate analyses and Mantel tests indicate the presence of a significant east–west geographic gradient. Analyses of molecular variation suggest that most of the observed variation occurs within taxa, rather than among taxa. A weak inter-taxon variation is nonetheless significant for RAPD and ISSR data, and a weak pattern dependent on geographical location is evident within the province of Québec. In accordance with studies based on morphological characters, molecular data indicate that *R. rousseauiorum* and *R. williamsii* should not be considered as species distinct from *R. blanda*.

Key words: Rosa blanda, Rosa rousseauiorum, Rosa williamsii, Rosa johannensis, Rosa subblanda, RAPD, ISSR, AFLP, endangered plants, taxonomic status.

Résumé : *Rosa rousseauiorum* Boivin et *Rosa williamsii* Fern. sont deux rosiers rares, indigènes à l'est du Québec dont le statut taxonomique est controversé. Ils sont affiliés au *Rosa blanda* Ait. s.l. chez lequel un grand polymorphisme morphologique est observé. Une étude moléculaire qui vise à vérifier le statut taxonomique de ces deux taxons, ainsi que le statut de *Rosa subblanda* Rydb. et *Rosa johannensis* Fern., deux autres espèces ségrégées de *R. blanda* s.l., a été réalisée. Nous présentons des analyses de marqueurs RAPD, ISSR et AFLP sur 86 individus provenant de 36 populations de l'est de l'Amérique du Nord. Malgré un haut taux de polymorphisme intraspécifique, les analyses de groupement et d'ordination en espace réduit ne démontrent aucun regroupement correspondant aux espèces traditionnellement définies à l'intérieur de *R. blanda* s.l. Néanmoins, *Rosa palustris* Marsh., une espèce proche-parente servant de groupe témoin, est clairement distincte de *R. blanda* s.l. Lorsque des populations de l'ouest de l'aire de répartition de *R. blanda* sont incluses, un gradient est-ouest est observé et appuyé par l'ordination en espace réduit et des tests de Mantel. Les analyses de variance moléculaire suggèrent que la majorité de la variabilité génétique observée se trouve à l'intérieur des taxa plutôt qu'entre taxa. La faible variabilité inter-taxon est néanmoins significative pour les RAPD et les ISSR et un patron géographique faible regroupant les populations d'une même région est aussi observable au Québec. *Rosa rousseauiorum* et *R. williamsii*, difficilement différenciés à l'aide des caractères morphologiques, ne peuvent par les analyses moléculaires être distingués ni entre elles ni des autres espèces du complexe du *R. blanda* au Québec. Le statut taxonomique de ces deux espèces ne serait pas justifiable d'après ces données moléculaires.

Mots clés : Rosa blanda, Rosa rousseauiorum, Rosa williamsii, Rosa johannensis, Rosa subblanda, RAPD, ISSR, AFLP, plantes rares, statut taxonomique.

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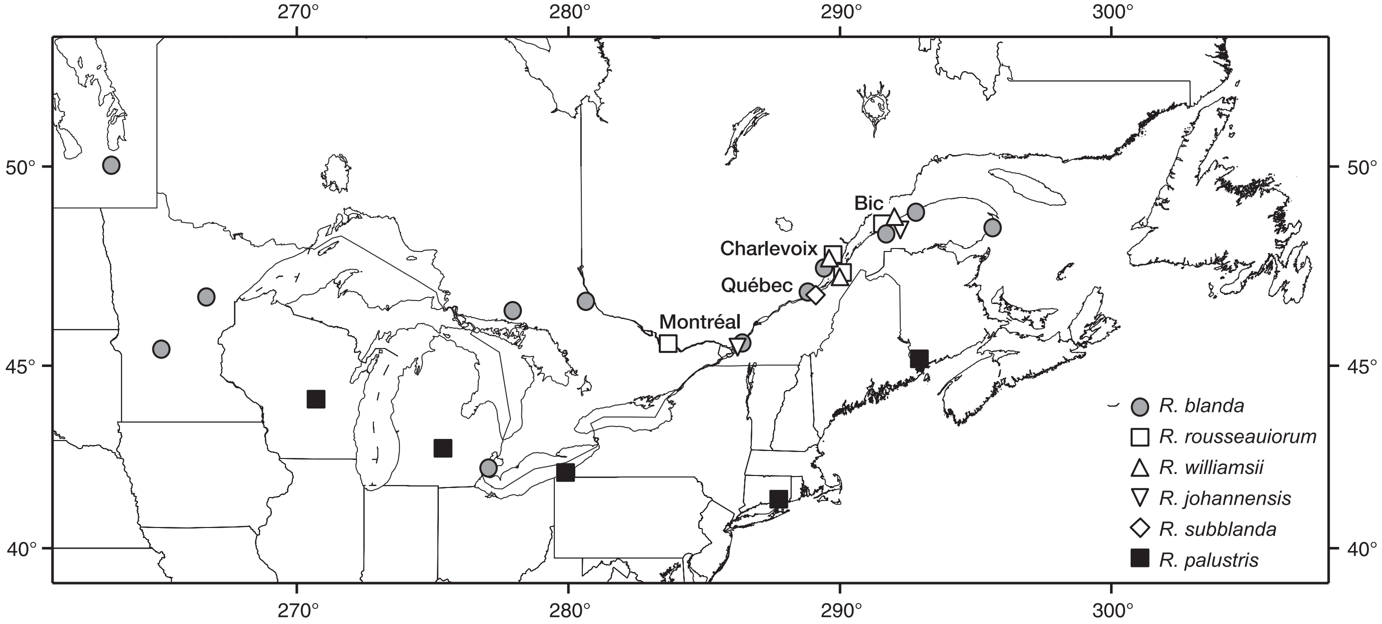
Fig. 1. Locations of sampled populations of *Rosa blanda* s.l. taxa and *Rosa palustris* in eastern North America. The four main areas of sampling in Québec (and those included in the AMOVA tests) are noted on the map, but not all populations within these areas are shown.



Introduction

Rosa blanda Ait. s.l. (*Rosa* section *Rosa*) is a common species in eastern North America that occurs from Nova Scotia to Saskatchewan, south to Pennsylvania and Missouri. *Rosa blanda* s.l. is a morphologically variable taxon in which numerous variants have been recognised at various taxonomic ranks, including several distinct species (Lewis 1957*b*). The taxonomic rank and status of all of these taxa are controversial. *Rosa blanda* and its segregates tend to occur in calcareous areas, whereas the other rose species native to eastern North America, *Rosa virginiana* Mill., *Rosa carolina* L., *Rosa nitida* Willd., and *Rosa palustris* Marsh., are generally found in more acidic soils (Fernald 1918). *Rosa blanda* s.l. is differentiated from these sympatric native species by the absence of infrastipular prickles, the presence of glabrous fruits, pedicels and peduncles, and by largely unarmed petioles and flowering branches. Within *R. blanda* s.l., variants have been recognised based on differences in sepal length, sepal orientation on the fruit (erect or not), shape and colour of the fruits, the degree of pubescence on the leaves and petioles, and the presence or absence of glandular trichomes on the dorsal surface of the stipules (Fernald 1918; Rydberg 1918; Erlanson 1934; Marie-Victorin and Rolland-Germain 1942; Boivin 1945; Scoggan 1978). However, Erlanson (1934) has shown that in controlled crosses, F_1 individuals can differ from their parents in the expression of these particular characters, and some of these characters are known to be polymorphic even within individuals (e.g., sepals erect or divergent at maturity), rendering controversial any taxonomic delimitation within *R. blanda* s.l. (e.g., Erlanson-Macfarlane 1966).

At the species level, four segregates of *R. blanda* s.l. sometimes have been recognised: *Rosa subblanda* Rydb., *Rosa williamsii* Fern., *Rosa johannensis* Fern., and *Rosa rousseauiorum* Boivin. Rydberg (1918) described the form with entirely glabrous leaflets as *R. subblanda*, a taxon that occurs sporadically across the range of *R. blanda* s.l., thus leaving the name *R. blanda* for the more typical form with pubescent or puberulent leaflets. This was meant to overcome the problem associated with the type description of *R. blanda* by Aiton (1789), which described the leaflets as glabrous, but apparently from a specimen different from the rest of the description (Fernald 1918; Lewis 1957*b*; Lysaght 1971). The other three species were described as variants having reflexed or divergent sepals at maturity, rather than the more common form in *R. blanda* of sepals erect at maturity (at the apex of the receptacle). *Rosa williamsii* was described by Fernald (1918) to account for a variant of *R. blanda* with glandular stipules that is narrowly restricted to the calcareous shores of the St. Lawrence River in Bic, Québec (Fig. 1). In 1945, Bernard Boivin described another glandular variant of *R. blanda*, *R. rousseauiorum*, a taxon that is distinguished from *R. williamsii* by its larger stature and longer sepals. In contrast to *R. williamsii*, *R. rousseauiorum* has a wider distribution ranging from shoreline habitats along the Gulf of the St. Lawrence to the lower reaches of the St. Lawrence River near Ottawa, Ontario. Fernald (1918) also described *R. johannensis*, a variant of *R. blanda* s.l. with glabrous leaflets, which occurs from Québec to New Brunswick, and south from Maine to northern New York. In addition, a number of forms, ecotypes, or varieties have at times been recognised (e.g., Crépin 1876; Schuette 1898; Fernald 1918, 1948, 1950; Rydberg 1918; Erlanson 1934; Boivin 1945; Lewis 1957*b*; Scoggan 1978). However, all of these segregate species remain controversial, with some authors (e.g., Breitung 1952) treating all four as synonymous with *R. blanda*. No consensus is yet available as to the taxonomic limits and rank of the *R. blanda* segregates, nor on which characters best distinguish the taxa in this morphologically variable taxon (Fernald 1918, 1950; Erlanson 1934; Boivin 1945, 1966; Breitung 1952; Scoggan 1978). Only the widespread *Rosa blanda* appears to have a non-controversial species status, but even the distinction between *R. blanda* and its western counterpart, *Rosa woodsii* Lindl., can be difficult to assess in zones where the two species overlap (Lewis 1957*b*, 1962).

The status of the two Québec endemics, *R. rousseauiorum* and *R. williamsii*, is of special interest. The restricted distribution of these two species to the St. Lawrence estuary (Charlevoix county and the lower St. Lawrence River), and the scarcity of recorded populations encountered, led botanists to list *R. williamsii* among the rare plants of Québec (Bouchard et al. 1983) and of Canada (Argus and Pryer 1990), and Lavoie (1992) added *R. rousseauiorum* to the list of threatened species in Québec. However, several botanists have questioned the validity of the specific status of these two taxa and thus the need for conservation priority. Because of their controversial and doubtful species status, they were removed from the list of potentially threatened and endangered species of Québec in the most recent survey (Labrecque and Lavoie 2002). However, it remains to be clearly demonstrated whether these taxa are taxonomically distinct from one another and from other forms of *R. blanda* s.l.

In the past decade, molecular methods based on the polymerase chain reaction (PCR) have made it possible to identify and generate fingerprints for cultivars of *Rosa* in the horticulture industry (Hubbard et al. 1992; Rajapakse et al. 1992; Vainstein and Ben-Meir 1994; Torres et al. 1993; Millan et al. 1996; Bédard 1997; Jan et al. 1999; Debener and Mattiesch 1999; Debener et al. 2000; von Malek et al. 2000; Crespel et al. 2002; Kaufmann et al. 2003). Such molecular tools also are appropriate for examining relationships among closely related plant species in nature (e.g., van de Wouw et al. 2001; Evans and Campbell 2002; Zhang and Kadereit 2002; Gustafson et al. 2003; Winfield et al. 2003) and have been shown to be powerful tools to help delimit species boundaries (e.g., Gobert et al. 2002; Ishida et al. 2003; Winfield et al. 2003). In this study we used random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), and amplified fragment length polymorphism (AFLP) markers to clarify taxonomic boundaries in *Rosa blanda* s.l., with particular emphasis on the region of Québec. The genus *Rosa* is notably complex taxonomically, and our study using molecular markers is a first attempt to clarify some of the taxonomic confusion that is encountered in North American roses. From a conservation perspective, the conclusions obtained in this study provide the necessary framework for deciding on conservation priorities, in as much as they help clarify the taxonomic status of *R. rousseauiorum* and *R. williamsii*, two taxa that could be considered threatened in North America.

Materials and methods

Sampling

A total of 86 individuals, collected from 36 populations and representing the four species segregate of *Rosa blanda* s.l. and *R. blanda* s.s., were studied (Table 1). The RAPD analyses were performed on 75 samples, the ISSR analyses were evaluated for 34 of these samples, while the AFLP markers were studied in 83 samples (Table 1). Because we started with the RAPD and ISSR analyses on a subset of the samples and only later added the AFLP analyses for a more thorough sampling scheme (these proved easier to implement), RAPD and ISSR analyses were not performed on all samples available.

We also included eight samples from five populations from throughout the range of *R. palustris*, another eastern North American species, to test the discriminatory ability of the AFLP markers (no *R. palustris* samples were examined with RAPD and ISSR markers). Preliminary analyses of the nuclear genome suggest that *R. palustris* is the sister group to the *R. blanda* – *R. woodsii* complex (S. Joly, unpublished data). The closely related *R. woodsii* is not an appropriate outgroup taxon because it hybridizes with *R. blanda* in the western portion of the distribution of this latter species.

Specimens for this study were collected primarily in the province of Québec, especially in the areas of Charlevoix and the lower St. Lawrence River, although samples from the western range of *R. blanda* (Ontario, Manitoba, and Minnesota) were also included (Table 1, Fig. 1). Samples from all known localities of *R. rousseauiorum* and *R. williamsii* were included in our study. Specimens were identified using the keys given in Boivin (1945) and Scoggan (1978). Specimens from Québec were carefully evaluated for stem armature, leaflet pubescence, presence of glandular trichomes on the stipules, sepal length, and whether sepals are erect or reflexed at maturity, characters considered to distinguish taxa within *R. blanda* s.l. In addition, because species identification in this group can be problematic, specimens also were identified independently by three other botanists (L. Brouillet, S. Hay, and J. Labrecque). This allowed us to arrive at a consensus regarding the identification of problematic specimens.

Molecular methods

Specimens collected in the field were preserved in silica gel prior to DNA extraction. DNA was extracted using the CTAB method described by Doyle and Doyle (1987), but with 1% polyvinyl-pyrrolidone, 1% β-mercaptoethanol, and 0.01 mol/L EDTA (pH 8.0) in the extraction buffer.

RAPD and ISSR analyses

RAPD and ISSR primers were initially selected based on the studies of Rieseberg (1996), Bédard (1997), and Wolfe and Liston (1998). Following an exploratory study on eight samples, RAPD primers OPA10, OPA11, OPC20, OPF13, and OPJ04 (Operon Technologies, Alameda, California) and ISSR primers 815, 821, 845, 849, and 859 (University of British Columbia, Vancouver, British Columbia) were retained because they showed the greatest amount of variation and gave the most reproducible results.

The RAPD amplification reactions included 1× PCR buffer (Roche Diagnostics; with 1.5 mmol/L $MgCl_2$ final), 200 nmol/L primer, 0.2 mmol/L of each dNTP, 2 U *Taq* DNA polymerase, approximately 40 ng DNA for a final volume of 25 μL. Amplifications were done in a Perkin-Elmer Gene Amp PCR System 9700 Thermocycler (Applied Biosystems (ABI), Foster City, California) using the 9600 emulation mode under the following conditions: 1 min dena-

Table 1. Specimens sampled and locality information for *Rosa blanda* s.l. and *Rosa palustris* analysed for RAPD, ISSR, and AFLP markers.

Voucher information	Collection locality	Geographical coordinates
***Rosa blanda* Ait. (*n*=42)**		
Bruneau 1205 (R, I, A)	Québec, Charlevoix, Les Éboulements	47°28′12″N, 70°20′24″W
Bruneau 1207 (R, I, A)	Québec, Charlevoix, Saint-Joseph-de-la-Rive	47°27′32″N, 70°21′32″W
Bruneau 1210a (R, I, A)	Québec, Montréal-est	45°35′48″N, 73°29′49″W
Bruneau 1210b (A)	Québec, Montréal-est	45°35′48″N, 73°29′49″W
Bruneau 1211 (R, I, A)	Québec, Montréal-nord	45°35′31″N, 73°38′20″W
Bruneau 1212 (R, I, A)	Québec, Montréal, Parc-de-la-Visitation	45°30′18″N, 73°50′02″W
Bruneau 1213 (R, I, A)	Québec, Montréal, Parc-de-la-Visitation	45°30′18″N, 73°50′02″W
Bruneau 1216 (R, I, A)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1217 (R, A)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1218 (R, I, A)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1219 (R, I)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1225 (R, I, A)	Québec, Ile d’Orléans, Saint-Jean	46°55′12″N, 70°53′20″W
Bruneau 1228 (R, I, A)	Québec, Ile d’Orléans, Saint-François	47°00′07″N, 70°48′47″W
Bruneau 1230 (R, I, A)	Québec, Ile d’Orléans, Saint-François	47°00′07″N, 70°48′47″W
Bruneau 1231 (R, I, A)	Québec, Ile d’Orléans, Saint-François	47°00′07″N, 70°48′47″W
Bruneau 1232 (R, I, A)	Québec, Ile d’Orléans, Saint-François	47°00′07″N, 70°48′47″W
Bruneau 1234 (R, A)	Québec, Bellechasse, Saint-Michel	46°52′26″N, 70°54′47″W
Bruneau 1235 (R, I, A)	Québec, Bellechasse, Saint-Michel	46°52′26″N, 70°54′47″W
Bruneau 1237 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1246 (R, A)	Québec, Bas St-Laurent, Rimouski	48°27′04″N, 68°31′37″W
Brunton 14115 (R, A)	Ontario, Petawawa	45°58′12″N, 77°20′24″W
Dignard 570 (R, A)	Québec, Gaspésie, Ile Bonaventure	48°29′24″N, 64 09′36″W
Dignard 574 (R, A)	Québec, Gaspésie, Ile Bonaventure	48°29′24″N, 64 09′36″W
Dignard 575 (R, A)	Québec, Gaspésie, Ile Bonaventure	48°29′24″N, 64 09′36″W
Drouin 98-016 (R, I, A)	Québec, Charlevoix, Baie Saint-Paul	47°26′27″N, 70°30′18″W
Drouin 98-017 (R, I, A)	Québec, Charlevoix, Baie Saint-Paul	47°26′27″N, 70°30′18″W
Drouin 98-018 (R, I, A)	Québec, Charlevoix, Saint-Joseph-de-la-Rive	47°27′32″N, 70°21′32″W
Drouin 98-020 (R, A)	Québec, Charlevoix, Ile-aux-Coudres	47°25′12″N, 70°23′24″W
Drouin 98-022 (R, I, A)	Québec, Charlevoix, Ile-aux-Coudres	47°22′48″N, 70°25′12″W
Drouin 98-023 (R, I, A)	Québec, Charlevoix, Ile-aux-Coudres	47°22′48″N, 70°25′12″W
Drouin 98-024 (R, I, A)	Québec, Charlevoix, Ile-aux-Coudres	47°22′48″N, 70°25′12″W
Joly 583 (A)	Ontario, Windsor	42°15′30″N, 83°02′59″W
Joly 584 (A)	Ontario, Windsor	42°15′30″N, 83°02′59″W
Joly 666 (A)	Minnesota, Jackson Co.	43°43′35″N, 95°03′50″W
Joly 668 (A)	Minnesota, Jackson Co.	43°43′35″N, 95°03′50″W
Joly 681 (A)	Minnesota, Pennington Co., Thief River Falls	48°06′36″N, 96°09′16″W
Joly 721 (A)	Manitoba, Birds Hill Provincial Park	50°00′59″N, 96°55′35″W
Joly 723 (A)	Manitoba, Birds Hill Provincial Park	50°00′59″N, 96°55′35″W
Joly 428 (A)	Manitoba, Birds Hill Provincial Park	50°00′56″N, 96°55′27″W
Joly 488 (A)	Ontario, Markstay-Warren Twp.	46°28′15″N, 80°29′27″W
Joly 590 (A)	Ontario, Markstay-Warren Twp.	46°28′15″N, 80°29′27″W
Saint-Laurent s.n. (R, A)	Québec, Bas St-Laurent, Sacré-Cœur	48°25′37″N, 68°35′27″W
***Rosa johannensis* Fern. (*n*=4)**		
Bruneau 1214 (R, A)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1215 (R, I, A)	Québec, Pierrefonds, Parc du Cap-Saint-Jacques	45°30′18″N, 73°50′02″W
Bruneau 1240 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 11 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
***Rosa rousseauiorum* Boivin (*n*=18)**		
Brouillet 99-23 (R, A)	Québec, Outaouais, Pontiac, Quyon	45°31′12″N, 76°13′55″W
Bruneau 1202 (R, I, A)	Québec, Charlevoix, Les Éboulements	47°28′12″N, 70°20′24″W
Bruneau 1204 (R, I, A)	Québec, Charlevoix, Les Éboulements	47°28′12″N, 70°20′24″W
Bruneau 1206 (R, I, A)	Québec, Charlevoix, Les Éboulements	47°28′12″N, 70°20′24″W
Bruneau 1239 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1243 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W

Table 1 (*concluded*).

Voucher information	Collection locality	Geographical coordinates
Bruneau 1206 (R, I, A)	Québec, Charlevoix, Les Éboulements	47°28′12″N, 70°20′24″W
Bruneau 1239 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1243 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1248 (R)	Québec, Bas St-Laurent, Saint-Fabien	48°19′01″N, 68°51′59″W
Bruneau 1250 (R)	Québec, Bas St-Laurent, Saint-Fabien	48°19′01″N, 68°51′59″W
Bruneau 1253 (R)	Québec, Bas St-Laurent, Rivière Ouelle	47°26′00″N, 70°03′06″W
Bruneau 1255 (R, A)	Québec, Charlevoix, Cap-aux-Oies	47°30′00″N, 70°14′24″W
Drouin 99-26 (R, A)	Québec, Charlevoix, Cap-aux-Oies	47°30′00″N, 70°14′24″W
Drouin 99-27 (R, A)	Québec, Charlevoix, Cap-aux-Oies	47°30′00″N, 70°14′24″W
Drouin 99-28 (R, A)	Québec, Charlevoix, Cap-aux-Oies	47°30′00″N, 70°14′24″W
Drouin 99-29 (R, A)	Québec, Charlevoix, Saint-Joseph-de-la-Rive	47°27′32″N, 70°21′32″W
Drouin 99-30 (R, A)	Québec, Charlevoix, Saint-Joseph-de-la-Rive	47°27′32″N, 70°21′32″W
Labrecque 9 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 10 (R)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 15 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
***Rosa subblanda* Rydb. (*n*=2)**		
Bruneau 1220 (R, I, A)	Québec, Ile d'Orléans, Saint-Laurent	46°51′36″N, 71°00′18″W
Bruneau 1227 (R, I)	Québec, Ile d'Orléans, Saint-François	47°00′07″N, 70°48′47″W
***Rosa williamsii* Fern (*n*=20)**		
Bruneau 1236 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1241 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1242 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1244 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1245 (R, I)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Bruneau 1249 (R)	Québec, Bas St-Laurent, Saint-Fabien	48°19′01″N, 68°51′59″W
Bruneau 1251 (R)	Québec, Bas St-Laurent, Saint-Fabien	48°19′01″N, 68°51′59″W
Bruneau 1252 (R, I, A)	Québec, Bas St-Laurent, Parc du Bic	48°19′01″N, 68°51′59″W
Bruneau 1254 (R, A)	Québec, Bas St-Laurent, La Pocatière	47°22′01″N, 70°02′24″W
Drouin 99-31 (R)	Québec, Charlevoix, Baie-Saint-Paul	47°26′27″N, 70°30′18″W
Drouin 99-32 (R)	Québec, Charlevoix, Saint-Iréné-les-Bains	47°33′00″N, 70°13′00″W
Labrecque 12 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 13 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 14 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 16 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 17 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 18 (R)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 19 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 20 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
Labrecque 22 (R, A)	Québec, Bas St-Laurent, Parc du Bic	48°21′36″N, 68°45′36″W
***Rosa palustris* Marsh. (*n*=8)**		
Joly 426 (A)	New Brunswick, York Co.	45°16′39″N, 67°28′42″W
Joly 430 (A)	New Brunswick, York Co.	45°16′40″N, 67°28′49″W
Joly 476 (A)	Connecticut, Stonington Co.	41°20′43″N, 71°54′14″W
Joly 477 (A)	Connecticut, Stonington Co.	41°20′43″N, 71°54′14″W
Joly 569 (A)	Pennsylvania, Erie Co.	42°09′33″N, 80°07′11″W
Joly 587 (A)	Michigan, Jackson Co.	42°19′32″N, 84°29′51″W
Joly 590 (A)	Michigan, Jackson Co.	42°19′32″N, 84°29′51″W
Joly 794 (A)	Wisconsin, Adams Co.	44°01′31″N, 89°43′13″W

Note: Following the voucher information we indicate whether samples were studied for RAPD (R), ISSR (I), or AFLP (A) markers. All vouchers are deposited at MT.

turation at 94 °C, 1 min annealing at 40 °C, and 2 min extension at 72 °C, for 45 cycles, and a final extension of 10 min at 72 °C ended the programme. A ramping of 25% was applied between the annealing and extension phases to increase binding efficacy and thus increase the reproducibility of the results.

For the ISSR analyses, the amplification reaction contained 1× PCR buffer (Roche Diagnostics, Laval, Quebec;

with 1.5 mmol/L $MgCl_2$), 600 nmol/L primer, 0.2 mmol/L of each dNTP, 2 U *Taq* DNA polymerase, approximately 75 ng DNA for a final volume of 25 µL. Amplifications were done using the same thermocycler as for the RAPD analyses, under the following conditions: 90 s denaturation at 94 °C, followed by 36 cycles of 90 s denaturation at 94 °C, 45 s annealing at 40 °C and 90 s extension at 72 °C. A final extension of 5 min at 72 °C ended the programme. A ramping of 33% was applied.

For each RAPD and ISSR primer, amplifications were repeated two to three times in identical conditions to identify reproducible amplification fragments. Amplification products were migrated and visualized on 1.5% agarose gels using ethidium bromide. Two commercial DNA markers (λ*Hin*dIII from Promega, Madison, Wis., and DNA molecular weight marker XIV from Roche Diagnostics) were used to ascertain fragment length.

AFLP analysis

The AFLP analyses were done using the protocol for large genomes recommended by ABI, but with certain modifications. Genomic DNA (300 ng) was digested with two restriction enzymes, *Eco*RI and *Mse*I (New England Biolabs, Pickering, Ontario), and ligated to double-stranded *Eco*RI and *Mse*I adapters (ABI) in a single step at 37 °C for 3 h. The reaction mix, in a final volume of 11 µL, contained 0.25 U T4 DNA ligase (Roche Diagnostics), 1× T4 ligase buffer (Roche Diagnostics), 1 µL of *Mse*I (50 µmol/L) and *Eco*RI (5 µmol/L) adapters, 5 U *Eco*RI, 1 U *Mse*I, 0.55 µL of BSA (1 mg/mL), and 1.1 µL of NaCl (0.5 mol/L). The product of the restriction–ligation reaction was diluted 20-fold with a $TE_{0.1}$ buffer (20 mmol/L Tris, 0.1 mmol/L EDTA, pH 8.0). Pre-selective amplifications were performed in the same thermocycler as for the RAPD and ISSR analyses under the GeneAmp 9600 emulation mode for ramping speed. The reaction mix contained 1× PCR buffer (Roche Diagnostics), a total of 3 mmol/L of $MgCl_2$, 300 pmol/L of *Eco*RI and *Mse*I +1 primers, 200 pmol/L of each dNTP, 1.6 U *Taq* DNA polymerase, and 4 µL restriction–ligation (or pre-selective) dilution in a 20-µL reaction volume. Following the pre-selective amplification, the product was diluted 20-fold in $TE_{0.1}$. Pre-selective amplifications were done under the following conditions: 2 min extension at 72 °C, followed by 20 cycles of 30 s denaturation at 94 °C, 30 s annealing at 56 °C, and 2 min extension at 72 °C, with a final extension of 30 min at 60 °C.

A total of 18 primer combinations containing an *Mse*I primer and three *Eco*RI primers (either *Eco*RI-ACA (blue), *Eco*RI-AAG (green), *Eco*RI-AAC (yellow)) were initially tested on 16 samples. Three combinations were chosen because they showed the greatest amount of variation across species in these initial assays: *Mse*I-CTA + *Eco*RI-AAC (yellow), *Mse*I-CAC + *Eco*RI-AAG (green), *Mse*I-CAC + *Eco*RI-ACA (blue). Selective (+3) amplifications were done following the ABI protocol, with ramping at 90% under the 9600 GeneAmp emulator on the 9700 GeneAmp thermocycler. Following the selective amplification, 0.5 µL of each combination was pooled together with 12 µL Hi-Di formamide (ABI) and 0.15 µL GeneScan-500 ROX size standard (ABI), and denatured for 5 min at 95 °C. Samples were run on an ABI 3100 automatic sequencer.

AFLP fragments were scored using the programme GENOGRAPHER (version 1.6, Montana State University, http://hordeum.msu.montana.edu/genographer/). Raw data files were imported into GENOGRAPHER and aligned by size between 35 and 500 bp using the internal standard. AFLP fragments between 50 and 500 bp were evaluated and scored.

Data analysis

RAPD, ISSR, and AFLP amplification fragments were scored as present or absent, and the data were coded into a binary data matrix. Only unambiguous fragments that were distinct and reproducible were coded for all three marker types.

The binary presence or absence matrices were analysed using the R Package (version 4.0d8; Casgrain and Legendre 2001). The raw data were converted into similarity matrices using Jaccard's coefficient and then into distance matrices ($D = 1 - S$). Jaccard's coefficient was used because it does not take into account double absences in pair-wise comparisons. For each of the matrices, Shepard diagrams were generated in the R package for two and three axes of variation to determine if the distance relationships between individuals was well represented in a reduced space. The distance matrices were then subjected to a principal coordinate analysis (PCoA) using the R package. Genetic variation was observed for the three principal coordinates, both with and without the inclusion of *R. palustris* samples (for the AFLP analysis). PCoA were performed for the RAPD, ISSR, and AFLP matrices, as well as for a combined matrix that included fragments from all three primer types (for 31 individuals). Similarly, the weighted pair group method with arithmetic averaging (WPGMA), which accounts for unequal and non-systematic sampling (Legendre and Legendre 1998), was used to generate phylograms for each of the same four matrices, using the R package.

Additional analyses were conducted to evaluate the compartmentalisation at different hierarchical levels of the total genetic variation observed using an analysis of molecular variance (AMOVA; Excoffier et al. 1992). We compared genetic variation between *R. rousseauiorum* and *R. williamsii* and the remaining species of *R. blanda* s.l. in Québec, and among the five *R. blanda* s.l. taxa, and among four geographic regions in the province of Québec (Montréal, Québec, Charlevoix, Bic; Fig. 1). These four regions were designated because they included the largest number of individuals in a confined geographic region. For the AFLP analyses among taxa, comparisons were done excluding *R. subblanda* because we had only one sample for this taxon. The AMOVA analyses were implemented in ARLEQUIN (version 2; Schneider et al. 2000) on the distance matrices derived from Jaccard's coefficient (see above). Statistical significance of the results was tested on 10 000 permutations.

Mantel tests were applied to the data to test for geographic structuring both the entire sampled range of *R. blanda* and within only Québec. The Mantel tests were implemented in the R Package on the Jaccard's coefficient derived distance matrices and on geographic distance (km) matrices derived from geographical coordinates. A total of

Table 2. Fragments amplified using RAPD, ISSR, and AFLP primers in a study of *Rosa blanda* s.l. in eastern North America.

Primer	Sequence (5′ to 3′)	Fragment size (bp)	Fragments scored	Monomorphic fragments[a]	Unique fragments[a]	Polymorphic (non-unique) fragments[a]
RAPD-A10	GTGATCGCAG	450 to 1100	36	10 (28%)	8 (22%)	18 (50%)
RAPD-A11	CAATCGCCGT	350 to 1400	13	0 (0%)	4 (31%)	9 (69%)
RAPD-C20	ACTTCGCCAC	900 to 1400	18	3 (17%)	4 (22%)	11 (61%)
RAPD-F13	GGCTGCAGAA	300 to 1300	37	6 (16%)	5 (14%)	26 (70%)
RAPD-J04	CCGAACACGG	700 to 1500	18	8 (44%)	7 (39%)	3 (17%)
ISSR-815	$(CT)_8G$	650 to 1500	7	3 (43%)	0 (0%)	4 (57%)
ISSR-821	$(GT)_8T$	750 to 1500	16	7 (44%)	2 (12%)	7 (44%)
ISSR-845	$(CT)_8RG$	950 to 1500	8	1 (12%)	2 (25%)	5 (63%)
ISSR-849	$(GT)_8YA$	800 to 1500	16	1 (6%)	5 (31%)	10 (63%)
AFLP-yellow	*Mse*I-CTA + *Eco*RI-AAC	56 to 446	61	7 (12%)	0 (0%)	54 (88%)
AFLP-green	*Mse*I-CAC + *Eco*RI-AAG	86 to 490	52	9 (17%)	5 (10%)	38 (73%)
AFLP-blue	*Mse*I-CAC + *Eco*RI-ACA	93 to 438	66	10 (15%)	3 (5%)	53 (80%)

[a] Percentages of total fragments that are monomorphic (non-variable), unique to particular individuals, or polymorphic among individuals are given in parentheses.

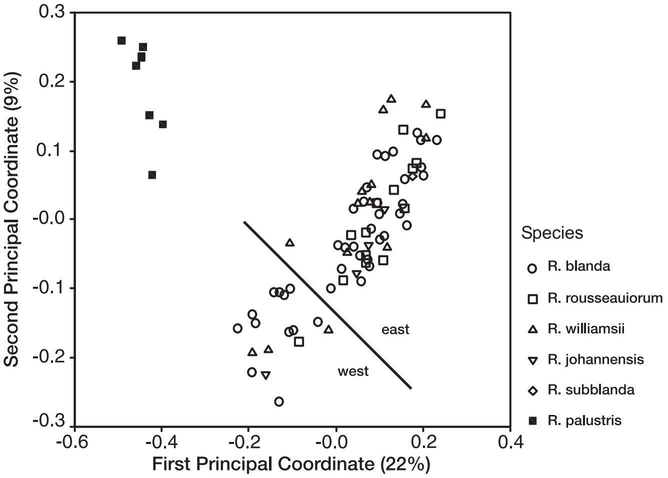
999 permutations were performed for each comparison (RAPD, ISSR, AFLP, all markers combined).

Results

The analysis of the five RAPD primers allowed us to identify 122 reproducible fragments for the 75 samples studied (Table 2). Of these, 77% were polymorphic (variable) among samples of *R. blanda* s.l. A low proportion of the fragments (23%) were unique to particular individuals, but of the non-unique polymorphic fragments only two were found exclusively in *R. blanda* s.s. and one exclusively in *R. williamsii*. Similarly, the four ISSR primers allowed us to discern 47 reproducible fragments for the 34 specimens analysed (Table 2), of which 81% were polymorphic. The ISSR analyses also yielded certain fragments unique to particular samples, but only a single polymorphic fragment each was found associated exclusively with *R. blanda* s.s. and *R. williamsii*. The three AFLP primer combinations yielded 179 unambiguous fragments for the 83 individuals analysed (Table 2). Of the 179 fragments scored, 85% were polymorphic, with only 4% of these unique to particular individuals. *Rosa palustris* was the only taxon that could be diagnosed by the presence or absence of polymorphic fragments unique to this taxon.

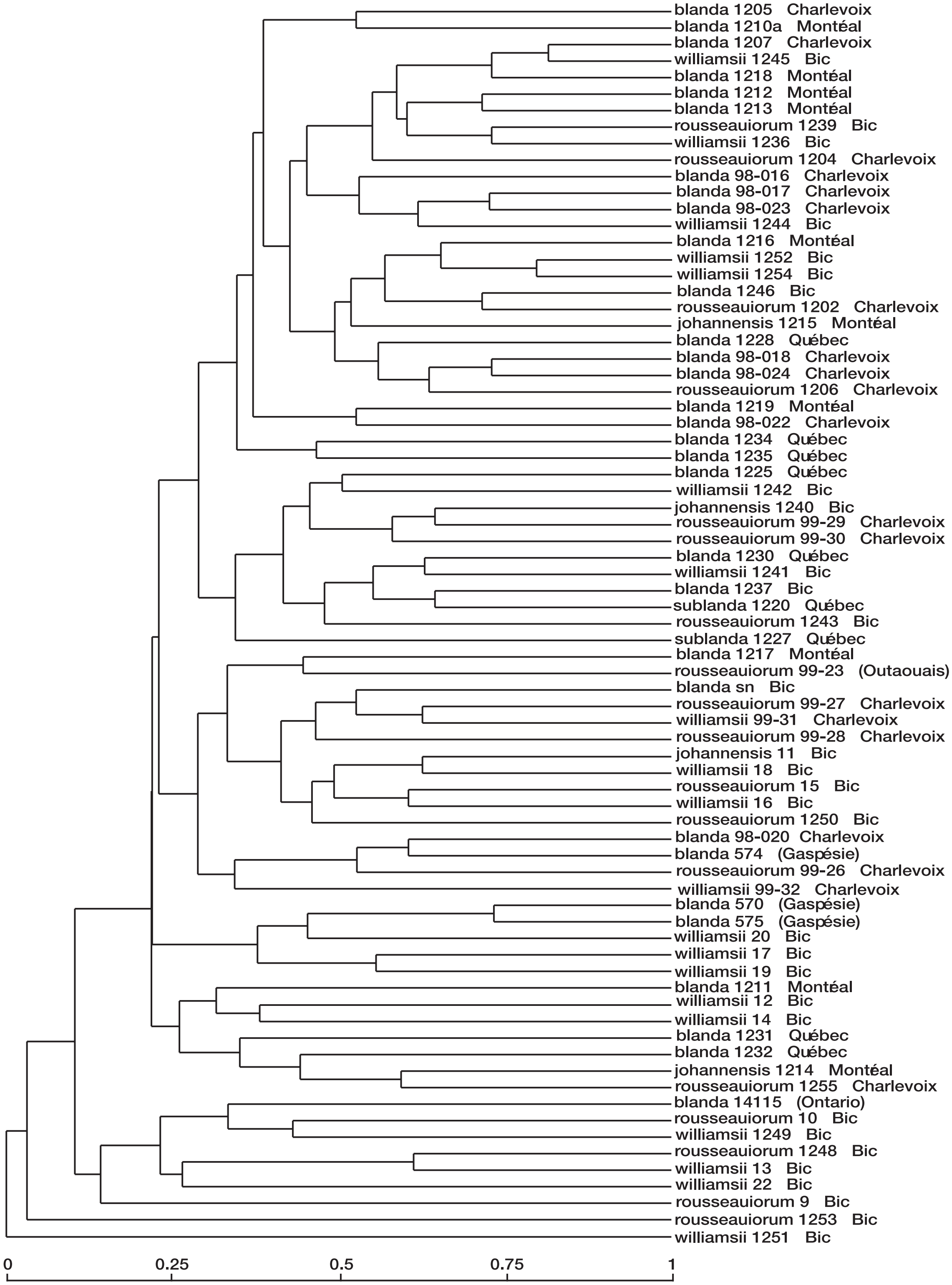
The Shepard diagrams (data not shown) for the four matrices (RAPD, ISSR, AFLP, combined) suggest that in general the distance relationships between individuals was well preserved in the reduced space, and found little difference between representations with two or three axes. The PCoA of the AFLP data clearly separates *R. palustris* from members of the *Rosa blanda* complex (Fig. 2). Within *R. blanda* s.l. the taxa are poorly differentiated. A weak east–west geographical gradient was apparent in *R. blanda* s.l. with most western populations occurring in the lower left corner of the graph and most eastern populations found in the upper right hand corner. This is supported by a significant and positive relationship between geographic distance and AFLP genetic distance within *R. blanda* s.l. as determined by the Mantel test (n = 76, r_M = 0.260, P = 0.005). The PCoA of the RAPD, ISSR, and combined data, which did not include western populations of *R. blanda* nor *R. palustris*, revealed a cluster of points for all the *Rosa blanda* s.l. taxa (results not shown). A similar pattern was observed in the WPGMA phylograms for the RAPD (Fig. 3) and AFLP (Fig. 4, including *R. palustris*) analyses. No groupings that correspond to either taxonomic delimitation or geographic regions could be discerned in these analyses. Similar results were obtained for both the ISSR and combined analyses (results not shown because of the reduced sampling regime). The absence of geographic structure in the molecular data was further demonstrated by the Mantel test showing the absence of a correlation between genetic and geographic distances within Québec for AFLP (n = 65, r_M = 0.044, P = 0.293), RAPD (n = 75, r_M = 0.092, P = 0.113), and combined (n = 31, r_M = 0.133, P = 0.106) data, although a slightly significant structure was observed for the ISSR data (n = 34, r_M = 0.229, P = 0.003).

Fig. 2. Principal coordinate analysis (PCoA) of AFLP markers in *Rosa blanda* s.l. and *Rosa palustris* in eastern North America, showing the first two axes, which account for 22% and 9%, respectively, of the total variance. The diagonal line indicates a division between most western and most eastern populations of *R. blanda* s.l.



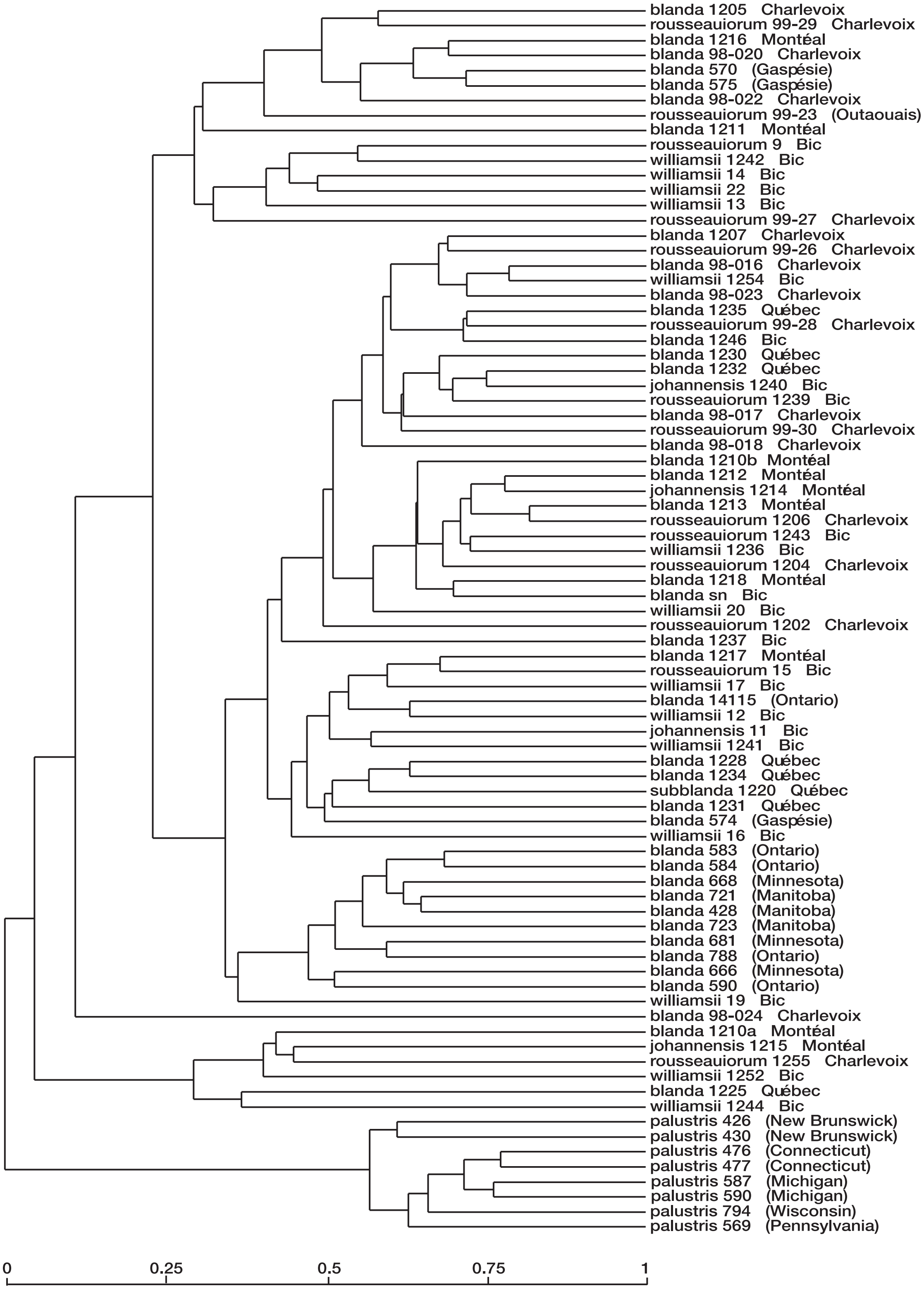
The partitioning of the genetic variation using an analysis of molecular variance (AMOVA) was evaluated to study pat-

Fig. 3. WPGMA phylogram of *Rosa blanda* s.l. taxa in the province of Québec based on the analysis of RAPD data. The locality for each sample is given after the species name. Localities in parentheses are those that were not included in the AMOVA tests.



terns of intra- and inter-taxon variation, and to study the possible presence of geographic structure in the molecular data within *R. blanda* s.l. In all of the analyses, the greatest amount of genetic variation observed is within taxa, rather than among taxa. The AMOVAs suggest little genetic differentiation among *R. blanda* s.l. taxa, and between the *R. rousseauiorum* – *R. williamsii* pair and the other *R. blanda* s.l. taxa (Table 3). Nonetheless, for the RAPD and ISSR, the low inter-taxa variation is statistically significant. Similarly a significant pattern of genetic differentiation

Fig. 4. WPGMA phylogram of *Rosa blanda* s.l. taxa and *Rosa palustris* in eastern North America based on the analysis of AFLP data. The locality for each sample is given after the species name. Localities in parentheses are those that were not included in the AMOVA tests.



among the four principal geographic regions of sampling within Québec was found for RAPD and ISSR markers but not for the AFLP data, even though most of the genetic variation occurs within regions (Table 3). The taxonomic and geographic partitioning of variance for the RAPD and ISSR data may not be entirely independent given that some taxa

Table 3. Hierarchical analysis of molecular variance (AMOVA) in *Rosa blanda* s.l. based on RAPD, ISSR, and AFLP analyses.

Source of variation	df	% total variance	*P* value
Among the five *Rosa blanda* s.l. taxa			
ISSR			
Among taxa	4	6.88	*P*(rand ≥ obs) = 0.017
Within taxa	29	93.12	
RAPD			
Among taxa	4	2.65	*P*(rand ≥ obs) = 0.002
Within taxa	70	97.35	
AFLP			
Among taxa	3	–11.18	*P*(rand ≥ obs) = 0.716
Within taxa	60	111.18	
Between the *Rosa rousseauiorum* – *Rosa williamsii* pair and other taxa			
ISSR			
Among two taxonomic groups	1	5.71	*P*(rand ≥ obs) = 0.206
Among taxa within two groups	3	2.98	*P*(rand ≥ obs) = 0.089
Within taxa	29	91.31	*P*(rand ≤ obs) = 0.023
RAPD			
Among two taxonomic groups	1	3.02	*P*(rand ≥ obs) = 0.198
Among taxa within two groups	3	0.46	*P*(rand ≥ obs) = 0.086
Within taxa	70	96.52	*P*(rand ≤ obs) = 0.003
AFLP			
Among two taxonomic groups	1	16.45	*P*(rand ≥ obs) = 0.664
Among taxa within two groups	2	–23.19	*P*(rand ≥ obs) = 0.781
Within taxa	60	106.74	*P*(rand ≤ obs) = 0.716
Among four geographical regions in Québec			
ISSR			
Among regions	3	15.16	*P*(rand ≥ obs) = 0.000
Within regions	30	84.84	
RAPD			
Among regions	3	5.01	*P*(rand ≥ obs) = 0.000
Within regions	64	94.99	
AFLP			
Among regions	3	–17.42	*P*(rand ≥ obs) = 0.790
Within regions	55	117.42	

Note: rand, random value; obs, observed value.

are strongly associated with a particular region (e.g., *R. williamsii* in the Bic region).

Discussion

Molecular markers and the delimitation of taxa within *Rosa blanda* s.l.

The RAPD, ISSR, and AFLP data yielded a high degree of genetic polymorphism among samples (77%, 81%, and 85%, respectively; Table 2), indicating that with these markers sufficient variation exists within *Rosa blanda* s.l. to group according to taxonomic boundaries. Despite this relatively high level of polymorphism, no groupings congruent with currently defined taxa were identified (Figs. 2–4), suggesting that little genetic differentiation exists among these taxa.

Roses are notoriously invariant in their genomes, often expressing greater morphological than genetic variation (Matsumoto et al. 1998; S. Joly and J.R. Starr, unpublished data). It is therefore important to identify molecular markers that are variable enough to reveal genetic, geographic, or taxonomic patterns. In the AFLP data set, the only analysis for which we had information on *R. palustris*, this close relative of *R. blanda* s.l. is clearly differentiated by molecular markers (Figs. 2 and 4). *Rosa palustris* is a diploid eastern North American rose that is quite distinct, morphologically, from *R. blanda* s.l. Nonetheless fertile hybrids between the two taxa have been thought to occur in nature (Erlanson 1934) and intermediate forms between the two have been named by various authors (Lewis 1957*b*). This suggests some genetic affinity and it also indicates that *R. palustris* may serve as a good reference taxon for testing the discriminatory ability of the markers applied to *R. blanda* s.l. Furthermore, the presence of a significant east–west geographic gradient within *R. blanda* s.l., as evidenced by the PCoA (Fig. 2) and Mantel tests, indicates that the markers used can detect some structure when present. Therefore, the absence of taxonomic grouping and weak geographic structure within Québec should not be interpreted as a problem with the molecular markers surveyed, but rather as a real observation needing an explanation. Similarly other studies have found such PCR-based markers to be useful in differentiating among closely related taxa (e.g., Gobert et al. 2002; Ishida et al. 2003; Winfield et al. 2003).

Taxonomic status of *Rosa rousseauiorum* and *R. williamsii*

Our data suggest that *R. rousseauiorum* and *R. williamsii* cannot be considered as species distinct from *R. blanda* s.s. Here we consider species to be ecologically, morphologically, and (or) genetically cohesive groups of populations that evolve independently from other such groups. The absence of cohesion is indicated by results of the PCoA (Fig. 2) and WPGMA (Figs. 3 and 4) analyses, as well as by the AMOVA, which suggests most of the genetic variation occurs within rather than among *R. blanda* segregates (Table 3). This suggests an important degree of gene flow among taxa within *R. blanda* s.l. in Québec. Field observations and morphological analyses further support the molecular data. Despite intensive collecting in the regions where *R. rousseauiorum* and *R. williamsii* are endemic, few specimens could be unambiguously identified as belonging to either of these two taxa, as described in the taxonomic key given by Boivin (1945).

Doubts have long persisted in the botanical community regarding the species status of *R. rousseauiorum* and *R. williamsii*, as well as that of *R. johannensis* and *R. subblanda*, the other two species that at times have been segregated from *R. blanda* s.l. Gleason and Cronquist (1991) recognised only *R. blanda* but mention *R. subblanda* and *R. johannensis* as possible varieties. In contrast, in *Gray's Manual of Botany* (Fernald 1950) and in the recent edition of *La Flore Laurentienne* (Marie-Victorin 1995), *R. blanda*, *R. johannensis*, *R. rousseauiorum*, and *R. williamsii* all are listed as good species. Similarly, Scoggan (1978) recognised *R. williamsii*, *R. rousseauiorum*, and *R. blanda*, the latter with two varieties and six forms. More recently, in *Flora of New Brunswick*, Hinds (2000) recognises only *R. blanda* (with *R. johannensis* as a synonym) but notes that a glabrous variety (var. *glabra* Crépin) is frequent in the region. Lewis (1957*a*, 1957*b*) in his taxonomic revision of North American roses did not recognise any of these species nor any infraspecific taxa, except for two forms: *R. blanda* Ait. f. *alba* (Schuette ex. Erl.) Fern. for a white-petaled variant and *R. blanda* Ait. f. *carpohispida* (Schuette) Lewis for a form with glandular–hispid hypanthia and pedicels. Likewise, Breitung (1952) in a study of native roses of Canada, recognised only *R. blanda*, describing the species as variable in terms of leaflet pubescence.

Rosa rousseauiorum and *R. williamsii* are distinguished from other *R. blanda* s.l. taxa by the presence of a large number of glandular trichomes on the lower surface of the stipules. Boivin (1945) described *R. rousseauiorum* to recognise large-stature plants that possessed stipules (>3.5 cm) and sepals (>1.5 cm) longer than those of *R. williamsii*. Unfortunately, the crucial differentiating character of sepal length is reversed in the discussion (i.e., <1.5 cm) relative to that given in the description and key (>1.5 cm), a situation that surely has added to the confusion associated with the taxonomic limits of *R. rousseauiorum* since its description. Although field observations support the idea that a population of *R. blanda* s.l. with glandular sepals and with plants of smaller stature does indeed occur in the Bic region of the lower St. Lawrence, as first suggested by Fernald (1918), we noted a high degree of variation in these characteristics. Plants can have more or less glandular stipules, often varying within a single individual, and sepal length likewise seems to represent a continuum from less than to greater than 1.5 cm. Lewis (1957*b*) also noted that across the range of *R. blanda* s.l., sepal length varied from 1.3 to 1.9 cm (mean 1.7 cm) and from the presence of glandular (47% of individuals measured) to non-glandular stipules. Similarly, leaflet pubescence was shown to vary, occurring in only 88% of specimens studied. In addition, Erlanson (1934) showed that individuals of *R. blanda* with erect sepals at maturity can have progeny with erect, spreading or sometimes reflexed sepals on mature hypanthia. All of these morphological analyses bring into doubt not only the taxonomic status of *R. williamsii* and *R. rousseauiorum*, but also of *R. johannensis*, *R. subblanda*, and most of the infraspecific taxa that have been described. Although our sampling for these other taxa is limited, our survey of molecular markers in specimens from Québec strongly suggests that on both molecular and morphological grounds, *R. blanda* should not be subdivided into several different species.

Biogeographical and conservation implications

The restricted geographical distribution of the *R. rousseauiorum* (Charlevoix and Lower St. Lawrence) and *R. williamsii* (Lower St. Lawrence River) variants suggests the possibility of a characteristic and well-defined biogeographic pattern within *Rosa blanda* s.l. in Québec. Molecular markers have been used in numerous phylogeographic studies to highlight such patterns (e.g., Tremblay and Schoen 1999; Abbott et al. 2000; Hagen et al. 2001; Stehlik 2002). Although in our analyses, no clear geographic pattern emerges from the PCoA, WPGMA analyses, or Mantel statistical tests, the AMOVAs suggest some partitioning of genetic variation relative to the four regions of Québec that we defined based on our sampling regime (Table 3). This suggests constraints on gene flow among regions in Québec, which may not be linearly related with the genetic distance and may therefore explain the lack of significance of Mantel tests. The St. Lawrence River may act as a barrier to gene flow across regions, resulting in differentiation between, for example, populations of Charlevoix, where most of the *R. rousseauiorum* variants occur, and Bic, where most of the *R. williamsii* variants are found. A stronger pattern of isolation by distance is evident when western *R. blanda* s.s. populations are included. Western *R. blanda* populations integrate with *R. woodsii* in regions where the ranges of the two species overlap (Manitoba, Minnesota, North and South Dakota) potentially adding increased genetic variability and differences to the western *R. blanda* gene pool (Lewis 1962; J.R. Starr, unpublished data). These analyses also suggest more gene flow among Québec populations than between Québec and the more western populations.

Both the *R. rousseauiorum* and *R. williamsii* variants tend to occur at the edge of the sea or in marsh habitats (Boivin 1945; Fernald 1950), but the latter seems to prefer a saline habitat (Fernald 1918). This led Erlanson (1934) to suggest that the latter taxon was a calciphile ecotype of *R. blanda* rather than a different species. Though restricted, the distribution of the *R. rousseauiorum* variant, in Charlevoix, the Gaspé Peninsula, the Bic region, and sporadically in the Gatineau valley, is nonetheless more widespread than that of the *R. williamsii* variant, which occurs almost exclusively in the Bic region (Fig. 1). Although there may indeed be a distinct morphotype of *R. blanda* in the Bic region with glandular stipules, small sepals, and an overall smaller stature, which merits conservation attention, all evidence suggests that *R. williamsii* should not be considered as a good taxonomic species. *Rosa rousseauiorum*, with its even less distinct phenotype and more widespread distribution, should simply be considered a synonym of the variable *R. blanda*.

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