stimulation, the snakes were maintained resting for 8 min and the blood samples were collected again as described.

The deproteinized blood samples were centrifuged at 16,000 × g for 2 min at 4 °C and the precipitate extracted 3 times with 6% perchloric acid. The lactic acid concentrations were determined in the pooled acid extract according to Marbach et al.[3] using lactate dehydrogenase purified from beef heart[4].

For the Bohr effect determinations, blood was collected from the left systemic arch into a heparinized syringe. The packed and washed erythrocytes were lysed as previously described[5]. After complete lysis, the solution was made 10^{-3} M with EDTA and centrifuged at 1200 × g. The oxygen-binding curves were obtained by a spectrophotometric method from the haemoglobin solutions with the pH adjusted from 6 to 8 by the addition of the following buffers: 0.01 M bis-tris-HCl pH 6–7 and 0.01 M tris-HCl pH 7–8 as described[2].

Results and discussion. The Hill plot of the data obtained for the 2 snakes is shown in the figure. The Bohr effect estimated from pH 7–8 in *H. modestus* and *L. miliaris* haemolysate were about −0.25 and −0.53 respectively, as judged by the equation $\Delta \log P_{50}/\Delta pH$. The table shows the results of lactic acid and Bohr effect determinations. It may be seen that *H. modestus*, the more aquatic species, presented haemoglobins with a lower Bohr effect and a blood lactic acid level of about 10 mg% in resting conditions, and 150 mg% after stimulation. The semi-aquatic snake, *L. miliaris*, on the other hand, presented a basal level of lactic acid of about 40 mg%, which increased after stimulation to 230 mg%. Its haemoglobin Bohr effect also showed higher values of −0.53 as compared to *H. modestus*.

In so far as our findings can be extrapolated to in vivo conditions, it seems possible that the higher Bohr effect values found in *L. miliaris* would allow the unloading of oxygen to the tissues even under relative acidosis conditions due to the low haemoglobin oxygen affinity at low pH, such as could be reached by the snake under stress situations as judged by the blood lactic acid content data. *H. modestus*, whose haemoglobin presents a lower Bohr effect, would not tolerate such acidosis conditions. Nevertheless, the lactic acid level would not attain such high values, as was observed with the blood lactic acid content of the snake submitted to stimulation, possible due to the lower levels of excitability of the more aquatic snake.

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The flavonoid glycosides of *Cornus canadensis* L. and its allies in Northwestern North America[1]

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Summary. The flavonoid glycoside profile of *Cornus canadensis* L. and its allies in Northwestern North America has been determined; quercetin 3-O-glucoside, 3-O-galactoside, 3-O-sophoroside and 3-O-gentiobioside; kaempferol 3-O-glucoside and 3-O-arabinoside. The discontinuity in distribution pattern of quercetin 3-O-gentiobioside within these taxa, associated with the phytogeography and historical factors affecting plant distribution in this area, indicates a possible polytopic and polychronistic origin of the hybrid members of the complex.

There are 3 commonly recognized species of herbaceous *Cornus* found in Northwestern North America[3]: *C. canadensis* L., *C. suecica* L. and *C. unalaschkensis* Ledeb. The latter causes considerable problems when found in the field as it is intermediate in morphology to both the other taxa. It has subsequently been referred to as a hybrid by various authors[4,5]. However Porsild[6] has reported collections of this taxon over 1000 miles from the nearest *C. suecica* Location, hence clouding the issue somewhat.

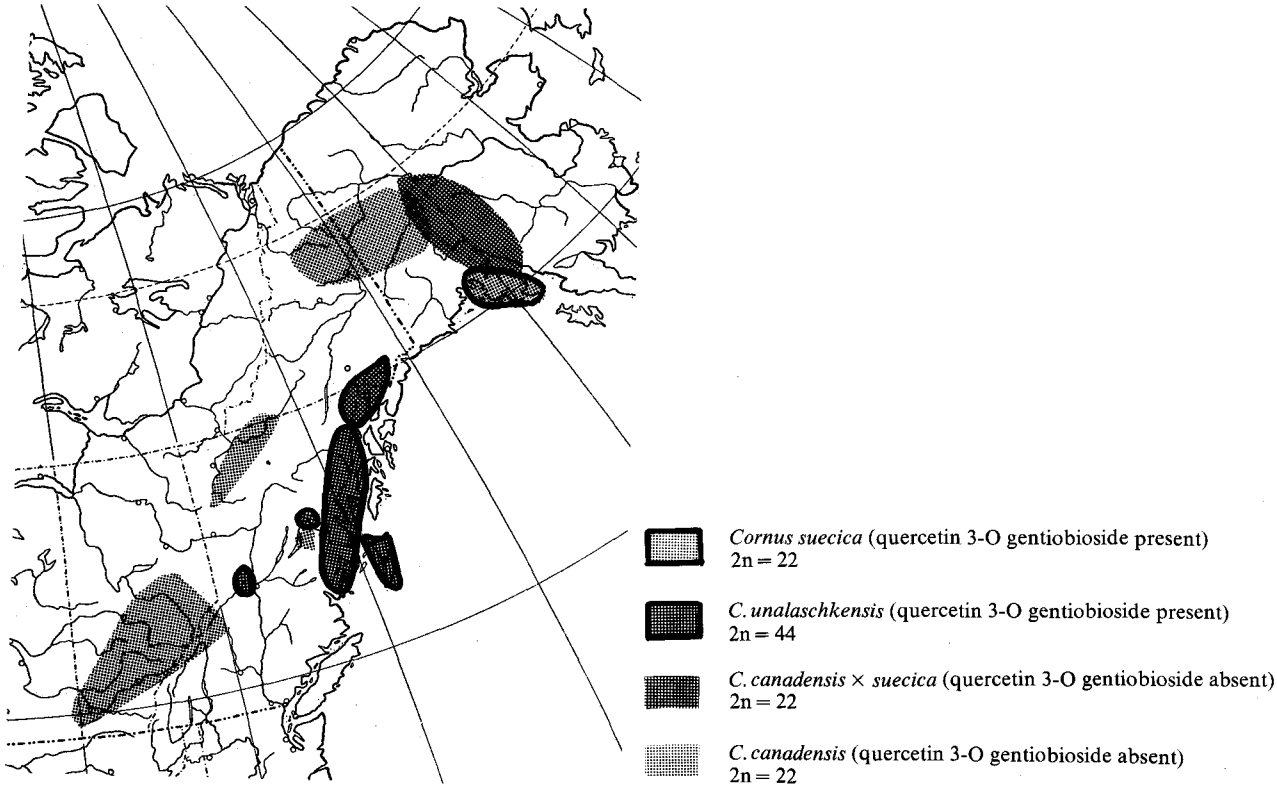
Previous studies[7–11] have shown that *C. suecica* and *C. canadensis* have chromosome counts of 2n = 22 and *C. unalaschkensis* 2n = 44. Studies in this laboratory and others have demonstrated that *C. unalaschkensis* is often confused with a semi-sterile intermediate with a chromosome number of 2n = 22 recognized as *C. canadensis* × *suecica* Hult[12].

Throughout Northwestern North America these 2 entities are indistinguishable from each other in the field and can only be categorically identified in the laboratory by statistical methods using guard cell measurements, chromosome numbers and pollen viability (*C. canadensis* × *suecica* having 50% viability as shown with lactophenol blue).

Previous flavonoid studies in this laboratory have been carried out successfully distinguishing between closely allied taxa[13–15] and an analysis of the flavonoid profiles of the 4 above taxa was undertaken to determine if chemical characters exist which would aid in the elucidation of taxonomic boundaries. A total of 48 samples of each taxon

Distribution of flavonoid glycosides in *Cornus*

Flavonoid glycoside	*Cornus Canadensis* 2n = 22	*Suecica* 22	*Canadensis* × *suecica* 22	*Unalaschkensis* 44
Quercetin 3-O-glucoside	+	+	+	+
Quercetin 3-O-galactoside	+	+	+	+
Quercetin 3-O-sophoroside	+	+	+	+
Quercetin 3-O-gentiobioside	−	+	−	+
Kaempferol 3-O-glucoside	+	+	+	+
Kaempferol 3-O-arabinoside	+	+	+	+



Distribution of the herbaceous *Cornus* used in present study.

were assayed for flavonoids. Air dried leaves of each were extracted with 80% ethanol and excessive chlorophyll removed with multiple aliquots of petrol-ether. 2-dimensional paper chromatography resulted in the purification and identification of 6 flavonol glycosides. Standard methods were used to establish flavonoid identities (UV, R_f's, fluorescence, spectrophotometry and direct comparison with known standards)[16].

The flavonoid profile of the *C. canadensis-C. suecica* complex is relatively simple. The presence of only flavonol glycosides indicates a modest chemical diversity. A major evolutionary step within the plant kingdom which has been shown to affect the flavonoid content of plants is the step from woody to herbaceous habit[17]. This produces 3 typical changes in leaf flavonoids: loss of protoanthocyanidins, loss of b-ring trihydroxylation, and replacement of flavonols by flavones. Because members of the *C. canadensis-C. suecica* complex are the only herbaceous members of an otherwise woody genus, it is therefore not surprising that they only exhibit 1 of the chemical changes usually associated with the morphological change from a woody to herbaceous habit. The loss of b-hydroxylation is shown by the lack of myricetin.

Of the 6 glycosides present, 5 are ubiquitous in distribution and hence of little taxonomic value. However quercetin 3-O-gentiobioside (spectral characters MeOH 250 (270), 359: R_f's BAW 35; Hw 17; acetic acid 28: sat. phenol 40), occurs only in *C. suecica* (2n = 22) and *C. unalaschkensis* (2n = 44). It is absent from *C. canadensis* and *C. canadensis* × *suecica* (both 2n = 22).

On examination of the distribution patterns of all 4 taxa investigated it can be seen that *C. suecica* and *C. unalaschkensis* both are essentially coastal in distribution existing in similar habitats (map), whereas *C. canadensis* and *C. canadensis* × *suecica* are non-coastal. The former 2 taxa appear to be restricted to suggested coastal nunataks and have a narrow distributional range than the latter. Such a pattern suggests a possible polychronistic and polytopic origin for these taxa. The restriction of the amphidiploid species to glacial refugia indicates it might have evolved during pre-glacial times, whereas the semi-sterile diploid hybrid probably is post-glacial in origin.

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