

may, under these circumstances, prevent males from locating females and thus reduce the incidence of mating and consequently the density of the pest population. The male brush-organ chemicals of the vine moth may therefore eventually provide an environmentally acceptable means of control once their behavioural significance is established.

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Haemoglobin Bohr effect and lactic acid content of the blood of 2 water-snakes with different degrees of aquatic adaptation¹

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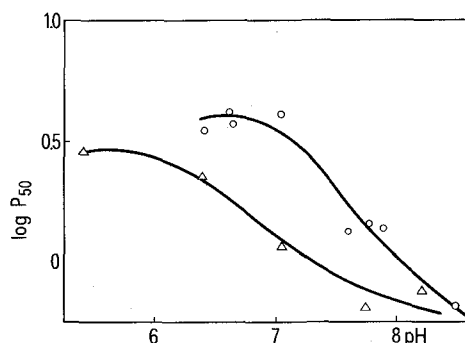
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Summary. *H. modestus*, a water-snake with morphological respiratory adaptation to its habitat, presents haemoglobins with a lower Bohr effect than those of *L. miliaris*, an aquatic snake without such respiratory adaptations. The difference in blood lactic acid content of the 2 snakes submitted to mechanical stimuli appears to be compatible with the properties of their haemoglobins.

Recently, 2 closely related species of water-snake have been studied in this laboratory². *Helicops modestus* is a sluggish water-snake with respiratory morphological adaptations and capable of diving for long periods of time. This snake presents haemoglobins which in the stripped form show a low Bohr effect (-0.07) and high oxygen affinity at pH 7 ($P_{50}=1.0$ mm Hg). *Liophis miliaris*, on the other hand, is a semi-aquatic snake without respiratory adaptations, and shows values of -0.3 for the Bohr effect and P_{50} of 1.41 mm Hg. These findings suggested that at low pH, the haemoglobins of *L. miliaris* would more readily unload oxygen to the tissues than those of *H. modestus*, and therefore the semi-aquatic snake would better tolerate acidosis conditions, such as those caused by the increase of the blood lactic acid when subjected to stimuli. To evaluate such a possibility, we submitted both snakes to mechanical

stimuli and determined their response and blood lactic acid content. We also analyzed the Bohr effect of the haemoglobins without treatment such as stripping in order to obtain values under conditions which could approach those found in vivo.

Material and methods. Adult forms of both *H. modestus* weighing 40-50 g and *L. miliaris* weighing about 100 g were placed individually in small plastic boxes at 25 °C for 12 h prior to blood collection. Blood samples of 0.1 ml were taken through a small incision in the snake's tail and added to tubes containing 6% perchloric acid. The snakes were then individually stimulated through mechanical aggression. During the stimulation, the snakes underwent an initial period of activity (5-6 min) after which they became completely exhausted and unresponsive to further stimuli. The response intensity of the less aquatic species, *L. miliaris*, was higher than that of *H. modestus*. After



Half-saturation oxygen tensions (P_{50}) of whole haemolysate haemoglobins of *H. modestus* (triangles) and *L. miliaris* (circles) as a function of pH. Buffers: 0.01 M bis-tris-HCl pH 6-7 and 0.01 M tris-HCl pH 7-8; temperature 25 °C; haemoglobin concentration 0.5-0.7 mg/ml.

Haemoglobin-oxygen equilibrium constants (P_{50}) at different pHs, Bohr effect and blood lactic acid concentration in *L. miliaris* and *H. modestus*. The alkaline Bohr effect is expressed as $\Delta \log P_{50}/\Delta pH$ and lactic acid concentration as mg % in the whole blood. 4 snakes of each species were used in the experiments

Determinations	pH	<i>H. modestus</i>	<i>L. miliaris</i>
$\log P_{50}$	6.5	0.30	0.60
	7.0	0.10	0.56
	7.5	-0.046	0.30
	8.0	-0.161	0.025
Bohr effect	-	-0.25	-0.53
Lactate level			
Undisturbed animals	-	10 ± 5*	40 ± 9
Stimulated animals	-	150 ± 13	230 ± 20

*SDE.

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 $16,000\times g$ 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 heart⁴.

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 described⁵. After complete lysis, the solution was made 10^{-3} M with EDTA and centrifuged at $1200\times g$. 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 described².

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 $\Delta\log P_{50}/\Delta\text{pH}$. 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 America¹

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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 America³: *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 Porsild⁶ has reported collections of this taxon over 1000 miles from the nearest *C. suecica* Location, hence clouding the issue somewhat. Previous studies⁷⁻¹¹ 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 chromo-

some number of $2n=22$ recognized as *C. canadensis* \times *suecica* Hult¹². 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* \times *suecica* having 50% viability as shown with lactophenol blue). Previous flavonoid studies in this laboratory have been carried out successfully distinguishing between closely allied taxa¹³⁻¹⁵ 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	<i>Cornus</i> <i>Canadensis</i> $2n = 22$	<i>Suecica</i> 22	<i>Canadensis</i> \times <i>suecica</i> 22	<i>Unalaschkensis</i> 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	+	+	+	+