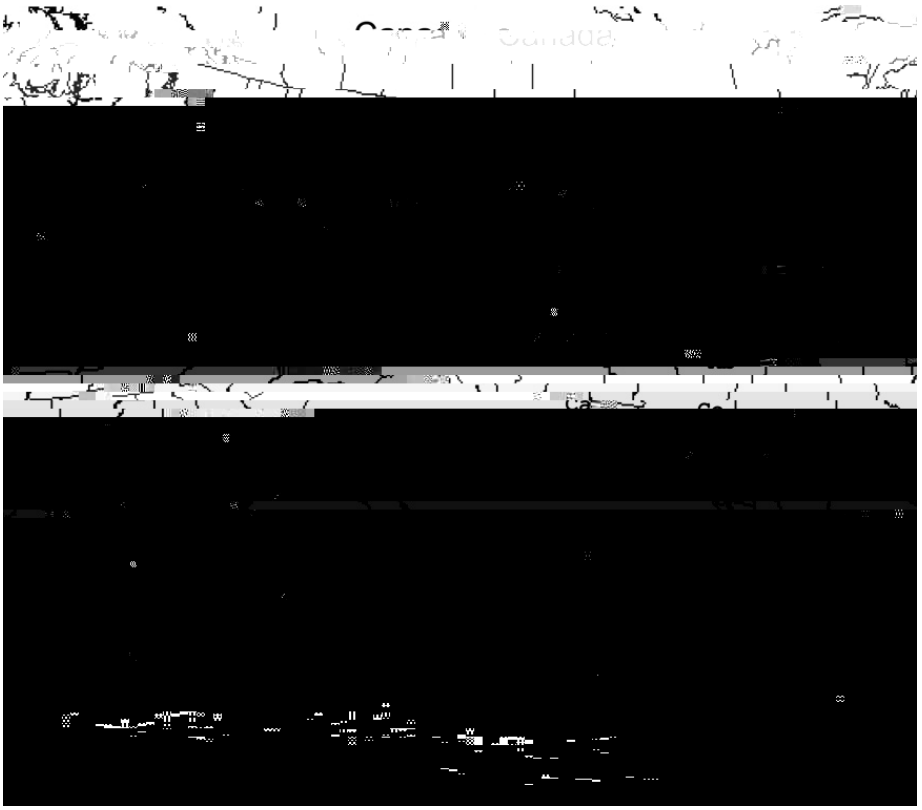
complex of both species, and the Coronado Island Rattle-



F . .1.

for approximately 2 h at 100 V; gels were then developed as described previously (Heussen and Dowdle, 1980; Munekiyo and Mackessy, 1998).

2.5. Mass spectrometry

Venoms from each subspecies were subjected to analysis using an ABI Voyager DE Pro matrix-assisted laser desorption/ionization-time-of- ight (MALDI-TOF) mass spectrometer operating in linear mode. Venoms (approximately 1.0 µg) were spotted onto sinapinic acid matrix (10 mg/mL 50% acetonitrile in water) and spectra were acquired in the mass range of 0.5–15 kDa.

2.6. Toxicity assays

Venoms were evaluated for intravenous lethal toxicity using female NSA mice weighing 25–30 g (3 mice per dose). All doses were adjusted to individual weights and delivered via the caudal vein in a bolus of 100 µL (0.9% saline), and toxicity was expressed as median lethal dose (LD_{50}). Procedures were conducted as approved by the UNC-IACUC (protocol 9401).

3. R . .

3.1. Venoms

Venom yields, snake masses and lengths, and general localities are shown in Table 1. Volume of venom yields increases exponentially with length as has been observed for other species (e.g., Mackessy, 1988; Mackessy et al., 2003), and the largest yields were obtained from the largest snakes.

3.2. Enzyme assays

Crude venoms were assayed for seven enzyme activities commonly found in rattlesnake venoms (Fig. 2). Thrombin-like and kallikrein-like serine protease activities were generally high in all venoms but were quite low in C. o. cerberus venom. Plasmin-like serine protease activity was

Subspecies

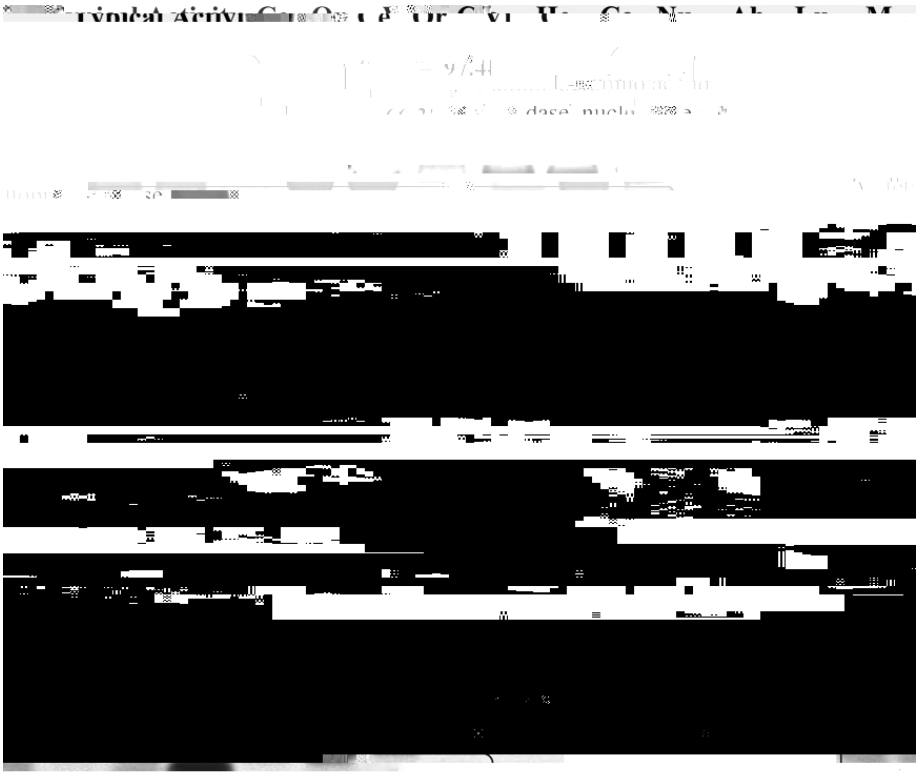


Fig. 3. Comparison of electrophoretic patterns of the nine nominate subspecies of the Crotalus viridis/oreganus complex on a bis-tris NuPage gel (24 μg venom/lane). Overall patterns are similar, but note the lack of PI and PIII metalloproteinases in C. o. concolor, C. o. caliginis and C. o. helleri venoms; differences in myotoxin amounts (band size and density) are also apparent among subspecies. Activities typical of bands of given masses are indicated. Abbreviations as in Fig. 1; M_r, Novex Mark 12 molecular weight standards (in kilodaltons).

generally low in all venoms except C. o. concolor and C. v. viridis venoms; activity was again lowest in C. o. cerberus venom. Metalloproteinase activity was generally high, but it was barely detectable in C. o. concolor venom and was very low in C. o. caliginis venom; activity was highest in C. o. cerberus venom.

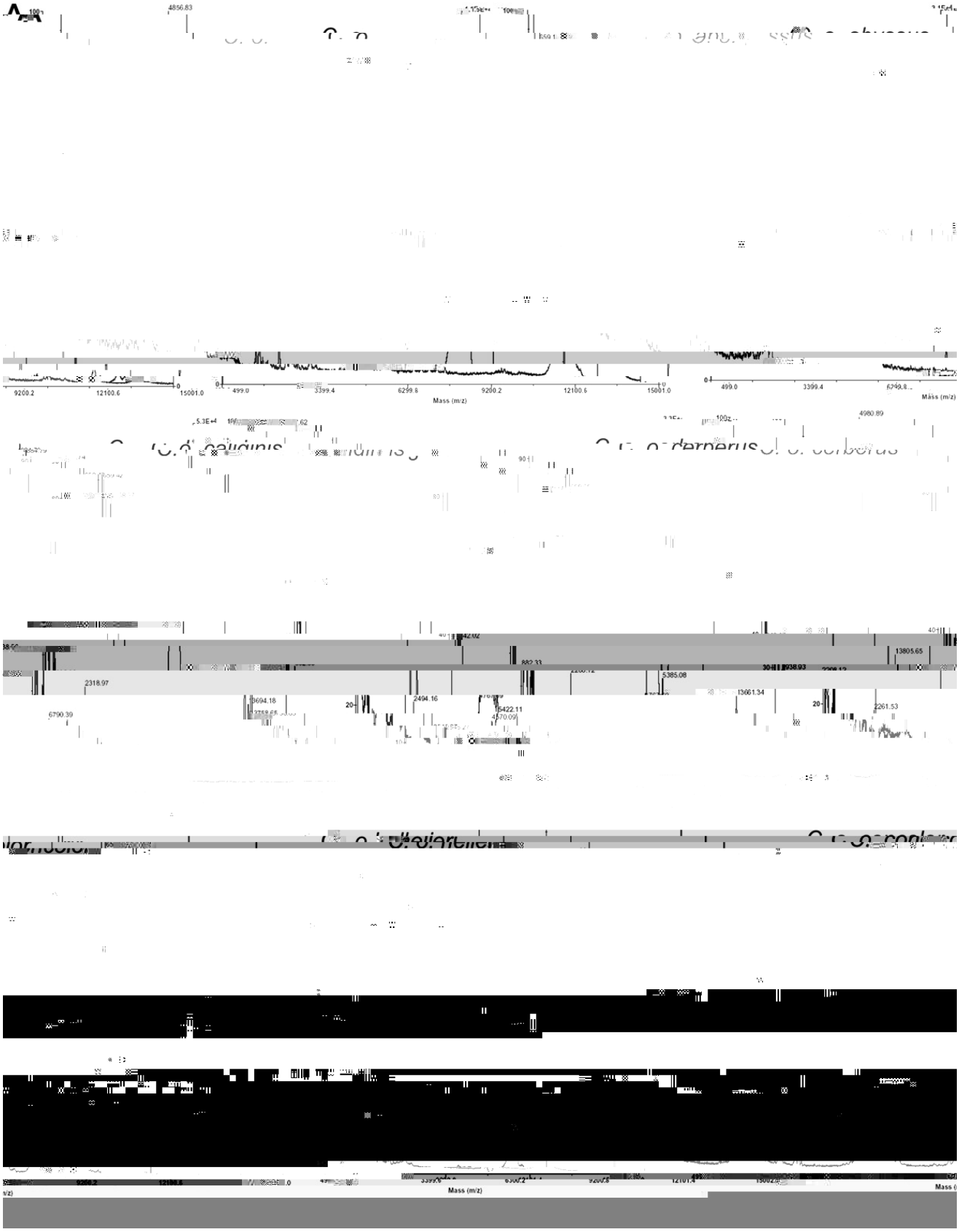
Phosphodiesterase levels were lowest in C. o. caliginis venom and high in C. o. abyssus, helleri and lutosus venoms. All venoms showed moderately high L-amino acid oxidase activity, and levels were highest in C. o. helleri venom. Phospholipase A_2 activity was highest in C. v. viridis venom and lowest in C. o. oreganus venom.

3.3. Electrophoresis

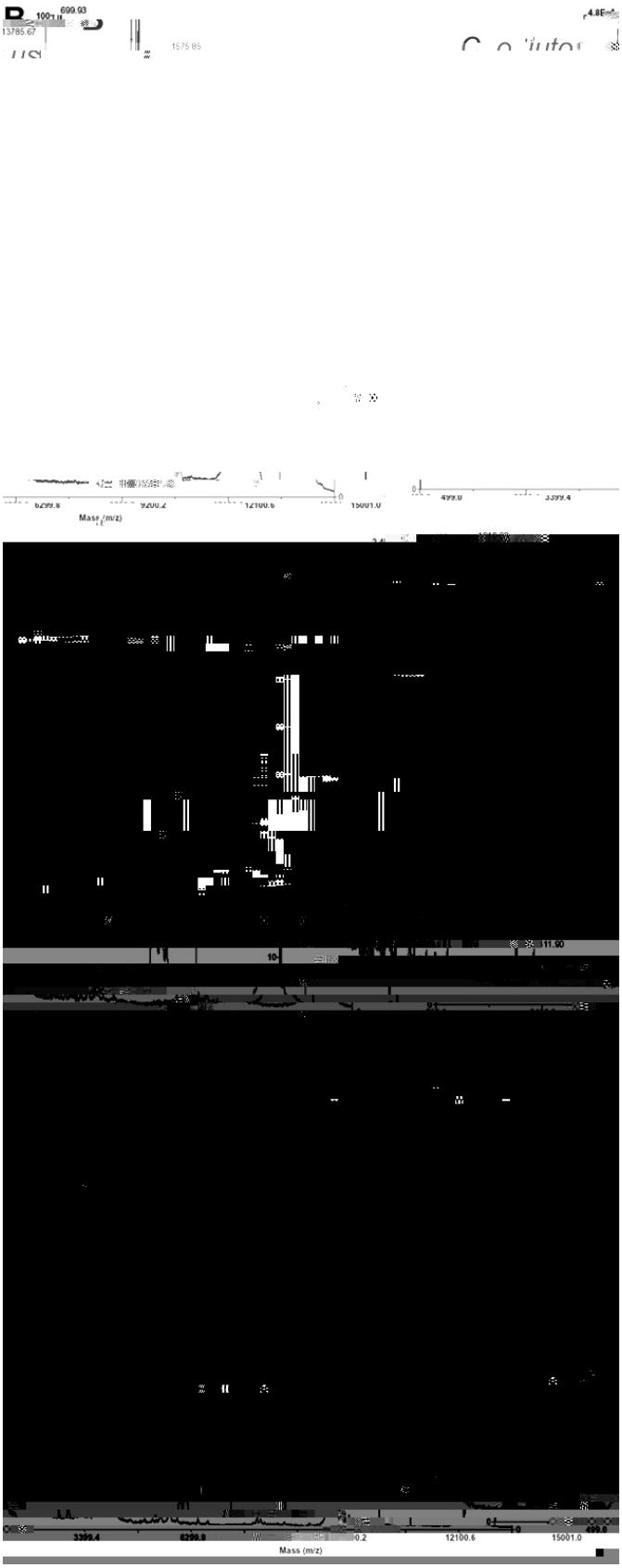
Electrophoresis on 12% acrylamide gels demonstrated the presence of 16–30 protein bands in the venoms (Fig. 3). General identification of bands was based on comparable migration of several purified enzymes (Mackessy, 1996, 2008, and unpubl. data) and on masses reported in the literature. Metalloproteinase bands were prominent in all venoms except C. o. concolor, C. o. caliginis and C. o. helleri venoms, concordant with results of enzyme assays. Phosphodiesterases and other nucleases, L-amino acid oxidase, serine proteases and phospholipase A_2s were present in all venoms, though apparent concentrations varied. Low molecular weight myotoxins (molecular weight ~4 kDa) were present in all venoms; levels were very high in C. o. caliginis, C. o. concolor, C. o. helleri and C. o. oreganus venoms and in low concentrations in venoms of C. o. cerberus, C. o. lutosus and C. v. viridis.

All venoms showed multiple metalloproteinase activities on zymogram gels (Fig. 4), typically with several of apparent molecular masses of 19–37 kDa and 53 kDa. A high molecular mass protease (approximately 74 kDa) was observed only in the venoms of C. o. cerberus and C. v.

Fig. 4. Metalloproteinase activity of venoms following zymogram electrophoresis on casein copolymerized gels; 0.5 μg venom was loaded in each lane. Protease activity appears as a clear band or region. High molecular weight proteases (~75 kDa) were seen only in C. o. cerberus and C. v. nuntius venoms, and C. o. concolor venom apparently contains only lower mass (<35 kDa) proteases. Venoms from C. o. oreganus, caliginis and helleri showed similar activity profiles. Abbreviations as in Fig. 1.



F . 5. MALDI-TOF mass spectra of venoms from the nine nominate subspecies of the Western Rattlesnake complex. Spectra were obtained on a Voyager DE MALDI-TOF mass spectrometer operating in linear mode with a window of 0.5–15 kDa (m/z).



F . 5. (continued).

nuntius, and only low molecular mass protease bands were seen in venom from C. o. concolor. It should be noted that mass estimates based on zymogram gels are somewhat inaccurate, so these values are most useful as relative masses. Metalloproteinase banding patterns of C. o. caliginis, C. o. helleri and C. o. oreganus were quite similar and were distinct from other subspecies.

3.4. Mass spectrometry

MALDI-TOF mass spectrometry of venoms revealed approximately 31 peptides with masses between 2 and 5.5 kDa in venoms of the nine nominate subspecies (Fig. 5; Table 2). In addition, approximately 26 peptides with masses of 6–15 kDa were observed in the same venoms (Fig. 5; Table 3). Peptide peaks with intensities of greater than 30% appear bolded in the tables, but it should be noted that this mass spectrometry method is not quantitative; some compounds (such as PLA_2s and CRISPs) ionize readily and efficiently, whereas others do not, so peak intensity is dependent on ionization efficiency rather than relative concentration. Peptides from different taxa were considered homologous (same line in Tables) if the masses were

Ses3 T30%)noakwD90272(the)-284422.5230Td[C.)1tly77 3oegan07(abyss6.3880

and de Queiroz (2001) has one of the most toxic venoms among rattlesnakes.

4. Discussion

Venoms of snakes serve several different biological roles, but biochemical features which enhance trophic functions are likely most important. Over most of its range,

degrading enhancement which facilitates ef cient digestion of the prey at suboptimal temperatures encountered in the eld. Most biological systems have inherent redundancies, and it was suggested previously that highly proteolytic activity of venoms allowed rattlesnakes and other temperate vipers to expand their foraging repertoire to include ambient conditions less than optimal (highly variable daily temperatures), essentially compensating for low temperature-induced compromise of gastrointestinal digestion (Mackessy, 1988). However, a very recent study suggested that there was no effect of envenomation on digestion ef ciency among two species of Trimeresurus (Chu et al., 2009), and we are currently re-testing the conclusions of Thomas and Pough (1979) using several species of temperate rattlesnakes and different thermal regimes.

Within C. oreganus, both extremes of venom compositional strategies (type I vs. type II) are observed, and either high metalloproteinase activity or high toxicity appears selected for in venoms. A general biochemical trend among venomous snakes is to produce either venoms rich in speci c toxins (commonly neurotoxic PLA_2s or three-

Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Press, Salt Lake City, UT, pp. 11–50.
Fox, J.W., Shannon, J.D., Stefansson, B., Kamiguti, A.S., Theakston, R.D.G., Ser S.M.T RCamargo.SC.M. N. 2U02.-320(RRol)-3244of discov19(e)r