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
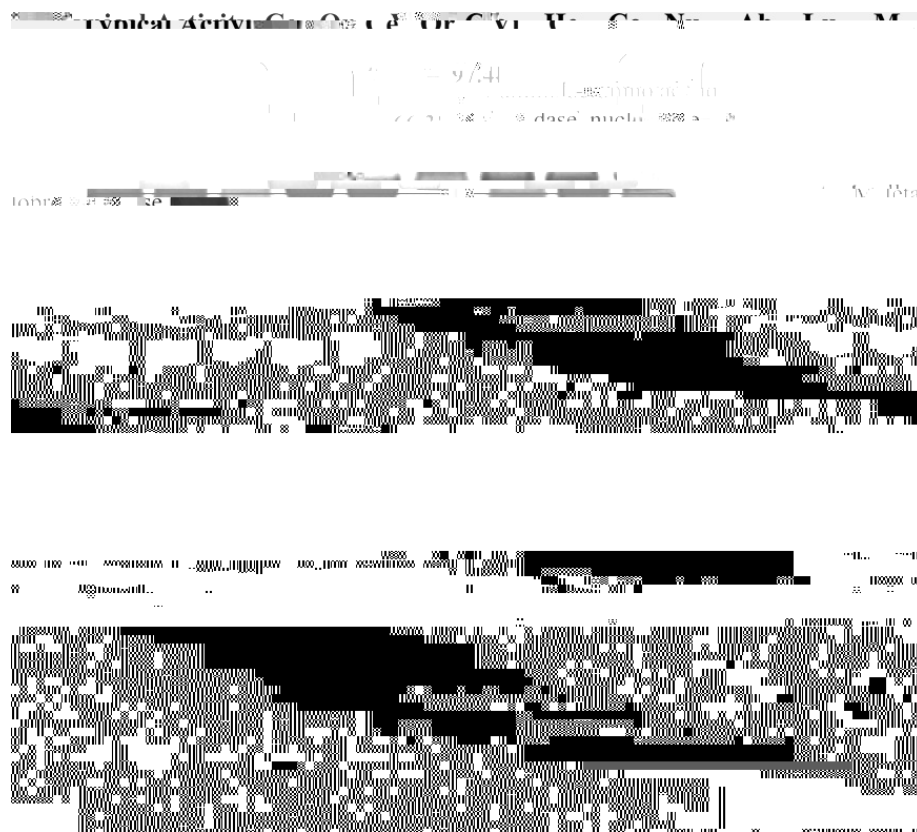
Fig. 3. Comparison of electrophoretic patterns of the nine nominate subspecies of the Crotalus viridis/oreganus complex on a bis-tris NuPage gel (24 μg venom/lane). Overall patterns are similar, but note the lack of PI and PIII metalloproteinases in C. o. concolor, C. o. caliginis and C. o. helleri venoms; 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

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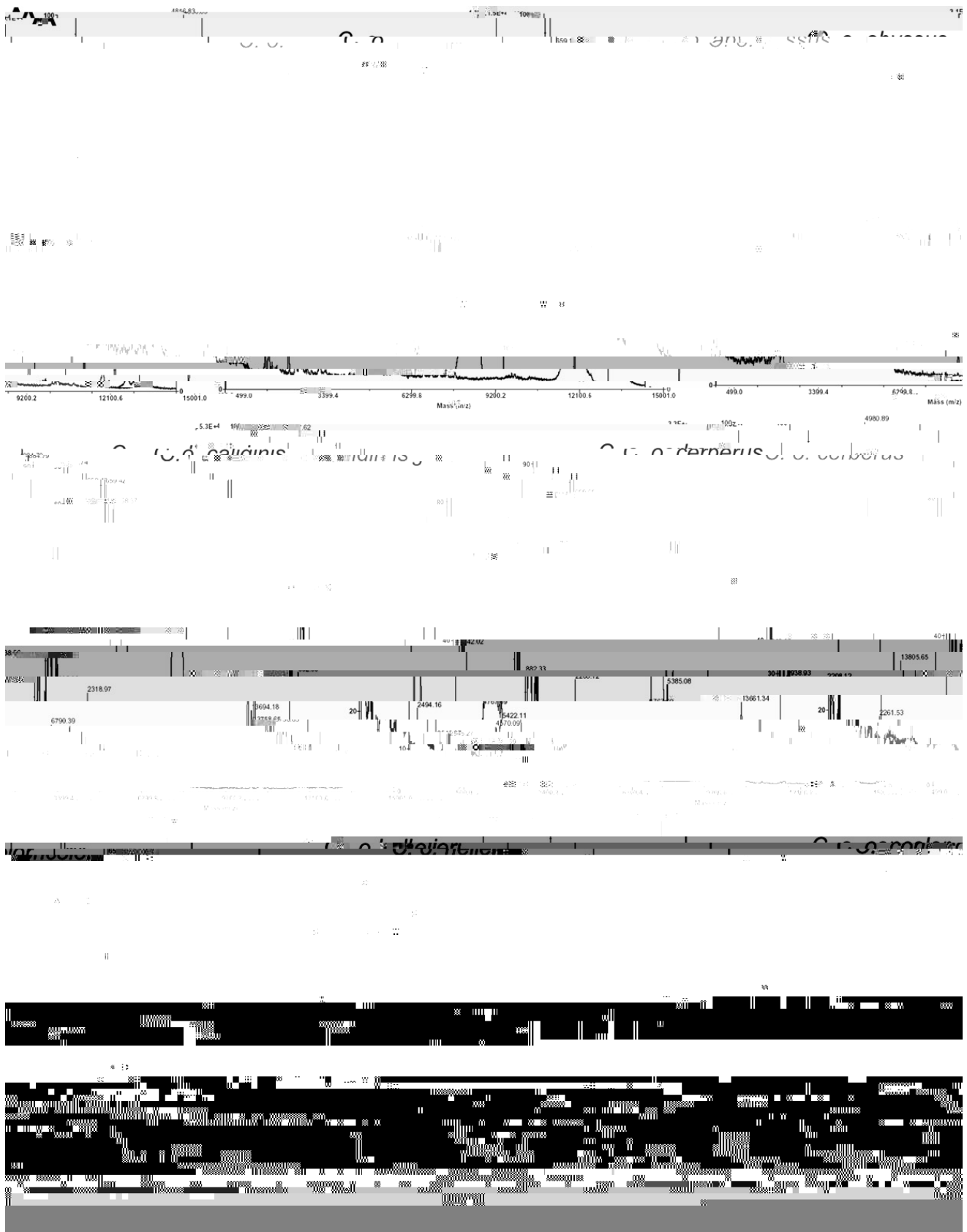
Fig. 5. MALDI-TOF mass spectra of venoms from the nine nominate subspecies of the Western Rattlesnake complex. Spectra were obtained on a Voyager DE MALDI-TOF mass spectrometer operating in linear mode with a window of 0.5–15 kDa (m/z).

Fig. 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$_2$s and CRISPs) ionize readily and ef ciently, whereas others do not, so peak intensity is dependent on ionization ef ciency rather than relative concentration. Peptides from different taxa were considered homologous (same line in Tables) if the masses were within 30%.

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

4. D c

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 diges-
tion of the prey at suboptimal temperatures encountered in
the eld. Most biological systems have inherent redun-
dancies, 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 composi-
tional 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