1998. P. A. Selden (ed.). *Proceedings of the 17th European Colloquium of Arachnology*, *Edinburgh 1997*.

The genetic population structure of *Euscorpius germanus* (C. L. Koch) (Scorpiones: Chactidae) in Switzerland

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Summary

We have examined the genetic population structure of *Euscorpius germanus* (C. L. Koch) in Switzerland, using horizontal starch gel enzyme electrophoresis (18 loci surveyed). We compared the allozyme data of *E. germanus* with data of three congeneric species: *E. carpathicus* (Linnaeus), *E. flavicaudis* (de Geer) and *E. italicus* (Herbst). The *E. germanus* populations were collected in five south-facing valleys of Switzerland (Mendrisiotto, Valle di Muggio, Val Bregaglia, Val Poschiavo and Val Müstair), and were expected to show moderate genetic differentiation because of their supposed postglacial colonization history and restricted dispersal abilities. However, allozyme analysis indicated a very low level of differentiation among populations of four valleys, whereas the eastern population from Val Müstair was highly differentiated. This suggests that the valleys were colonized from different refuges. Moreover, cluster analysis (UPGMA method using Nei's genetic distance as matrix) showed that the four *Euscorpius* species surveyed sorted out on an unexpectedly high genetic distance level. This is supported by including *Belisarius xambeui* Simon, *Buthus occitanus* (Amoreux) and *Mesobuthus gibbosus* (Brullé) as outgroup species.

Introduction

Scorpions vary in morphological and genetic patterns across their geographical ranges (Hadzi, 1931). Taxonomists traditionally defined variation among populations using patterns like hairiness, colour, number of pectinal teeth and trichobothria (Vachon, 1962; Ćurčić, 1972; Kinzelbach, 1975). Molecular methods are expected to contribute to our understanding of scorpion evolution. Among these methods, enzyme electrophoresis has provided a powerful tool for analysing patterns of genetic variation within and among populations and species, measuring heterozygosity, gene flow, and differentiation (Ferguson, 1980; Futuyma, 1986; Hartl, 1988). In a recent study of *Paruroctonus mesaensis* (Vaejovidae), Yamashita & Polis (1995a) showed that allozymes are potentially useful for the analysis of biogeographical problems. We therefore initiated a survey of the genetic structure of Swiss *Euscorpius germanus* populations (Chactidae) using horizontal starch gel electrophoresis of allozymes (18 enzyme loci surveyed).

Euscorpius germanus (C. L. Koch, 1837) (see Fet & Braunwalder, 1997) is found in the mountainous areas of northern Italy, Austria, Slovenia, Croatia, Bosnia and Bulgaria (di Caporiacco, 1950; Bonacina, 1980; Fet, 1993). In the northwestern part of its range, *E. germanus* is found in five south-facing valleys in Switzerland where this species occurs up to 2250 m (Alp Terza, Val Müstair) (Braunwalder & Tschudin, 1997). Most scorpion species have restricted dispersal abilities (Polis, 1990). Therefore, the populations of the geographically isolated Swiss valleys are expected to be separated and genetically differentiated at least since the colonization of these valleys after the last glaciation (10,000 yrs BP).

Enzyme	Abbreviation	EC	Buffer
Alanopine dehydrogenase	**ALPDH**	1.5.1.17	TBE
Arginine kinase-1	**ARK-1**	2.7.3.3	TBE
Aspartate aminotransferase-1	**AAT-1(GOT)**	2.6.1.1	TC
Aspartate aminotransferase-2*	**AAT-2(GOT)**	2.6.1.1	TC
Dihydrolipoamide oxidase	**DDH (DIA)**	1.8.1.4	AC
Glucose-6-phosphate isomerase	**GPI(PGI)**	5.3.1.9	TBE
Glutamate dehydrogenase	**GTDH**	1.4.1.2	TC
Glyceraldehyde-3-phosphate dehydrogenase	**GAPDH**	1.2.1.12	TBE
Hexokinase	**HK**	2.7.1.1	AC
Isocitrate dehydrogenase-1	**IDH-1**	1.1.1.42	AC
Isocitrate dehydrogenase-2*	**IDH-2**	1.1.1.42	AC
Malate dehydrogenase-1	**MDH-1**	1.1.1.37	AC
Malate dehydrogenase-2*	**MDH-2**	1.1.1.37	AC
Mannose-6-phosphate isomerase	**MPI**	5.3.1.8	TC
Peptidase	**PEP**	3.4.-.-	TC
Phosphoglucomutase	**PGM**	5.4.2.2	AC
6-phosphogluconate dehydrogenase	**6-PGD**	1.1.1.44	TC
Pyruvate kinase	**PK**	2.7.1.40	TBE

Table 1: The enzyme loci analysed and buffer systems used. Official abbreviations are indicated and other formerly known abbreviations are in parentheses. Mitochondrial and cytosolic forms are known for some gene loci, asterisks are given for enzymes which migrate cathodally (tissue: scorpion pedipalp).

For the evaluation of allozyme data on *E. germanus* populations, we have examined samples of three congeneric species, *E. carpathicus* (Linnaeus, 1767), *E. flavicaudis* (de Geer, 1778) and *E. italicus* (Herbst, 1800). Samples of *Belisarius xambeui* (Simon, 1879) (Chactidae: Euscorpiini Laurie, 1896), *Buthus occitanus tunetanus* (Herbst, 1800), *Buthus occitanus mardochei* (Simon, 1878) and *Mesobuthus gibbosus* (Brullé, 1832) (all Buthidae Simon, 1879) were used as outgroup species.

Material and methods

Sampling

Scorpions are easy to collect because of the fluorescence of their cuticle if illuminated with UV light (Sylvania F8T5/BLB). This collecting method was efficient for *E. carpathicus*, *E. flavicaudis* and *E. italicus*, but was not used for *E. germanus* because this alpine species is relatively small and inhabits crevices in steep hills. Sample sizes ranged from 6–24 animals and are listed in Table 2. In most cases, about ten individuals were collected in a circular area of about 100–300 m^2. Scorpions were transported live to the laboratory, then frozen and stored at –80 °C until used for electrophoresis.

E. germanus samples were taken from six sites in five south-facing valleys in Switzerland: Fornace 640 m (Mendrisiotto, Ticino); Rancate 350 m (Mendrisiotto, Ticino); Monte 680 m (Valle di Muggio, Ticino); Sotpunt 820 m (Val Bregaglia, Grison); San Carlo 1100 m (Val Poschiavo, Grison); Sta Maria 1400 m (Val Müstair, Grison); (Fig. 1). The other species were collected at the following sites: *E. carpathicus*: La Morra (Italy, Piemonte) and Mathis (France, Vaucluse); *E. flavicaudis*: Balazuc (France, Ardèche) and Lauris (France, Vaucluse); *E. italicus*: Coglio (Switzerland, Ticino) and Vico Morcote (Switzerland, Ticino);

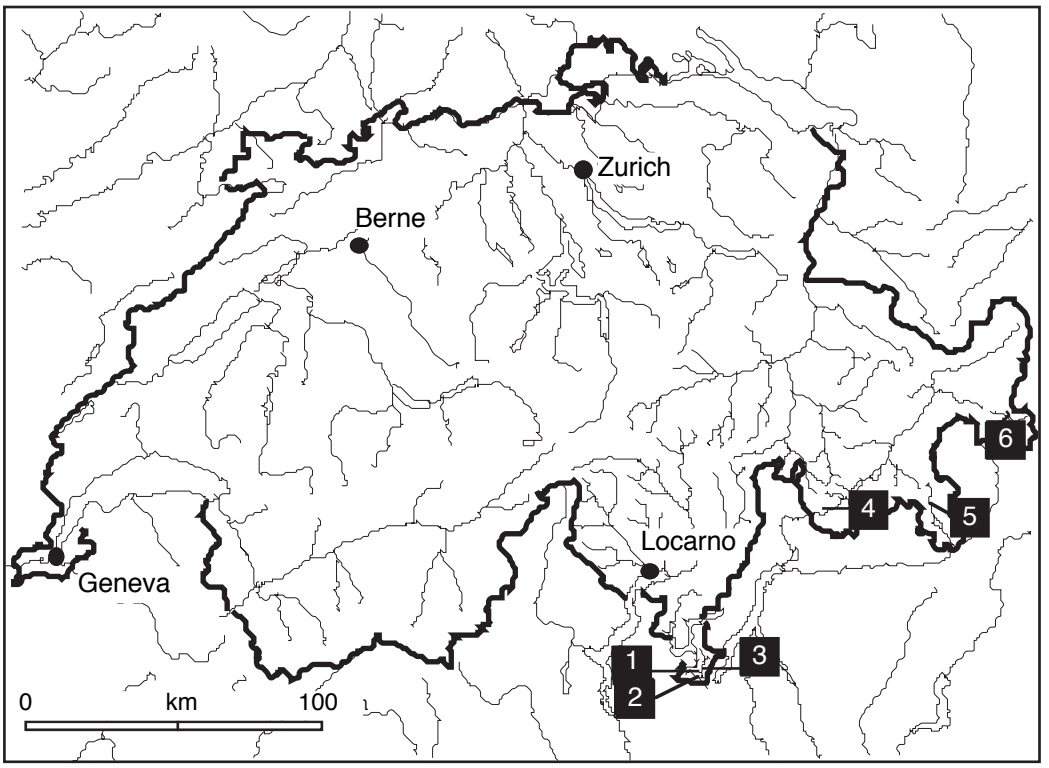

Fig. 1: Map of *E. germanus* sampling sites in Switzerland. 1 Fornace (Mendrisiotto); 2 Rancate (Mendrisiotto); 3 Monte (Valle di Muggio); 4 Sotpunt (Val Bregalia); 5 San Carlo (Val Poschiavo); 6 Sta Maria (Val Müstair).

B. xambeui: Amélie-les-Bains (France, Pyrénées-Orientales); *B. o. tunetanus*: (Tunisia, Tazoghrane, 2 specimens); *B. o. mardochei*: (Morocco, Agadir, 15 specimens); *M. gibbosus*: (Turkey, Central Anatolia, Ürgüp, 2 specimens).

Allozyme analysis

Tissue of the pedipalp was homogenized in ten volumes of buffer (Tris-HCl, 0.1 M pH 8.0); homogenates were then centrifuged for 5 min (13,800 G or 13,000 r.p.m.). 25 µl of supernatant fractions were applied to starch gels. We scored 18 gene loci on three buffer systems: N-(3-Aminopropyl)-morpholine-citrate (AC, pH 6.2), Tris-citrate (TC, pH 7.3), and Tris-borate-EDTA (TBE, pH 9.3). The buffer system which gave the best results for a locus was then used for routine analysis (Table 2). Enzyme stainings followed standard procedures (Harris & Hopkinson, 1976; Pasteur *et al*., 1988; Murphy *et al*., 1990) with minor modifications (Scholl *et al*., 1978). All zymograms were documented photographically (Polaroid).

We refer to the observed electromorphs as alleles which are identified by their electrophoretic mobility (in mm) relative to the most common mobility in the *E. flavicaudis* population from Lauris, France (assigned mobility = 100).

Statistical and phenogram analyses

Several parameters of genetic variation were calculated from the allozyme data (BIOSYS-1 program, Swofford & Selander, 1989). The variation within the populations was measured with the percentage polymorphism (0.95 criterion), the observed average heterozygosity, and the mean number of alleles resolved for a locus. The mean number of alleles per locus depends on the sample size (Nei, 1987a) and is therefore

		E. germanus						E. flavicaudis		E. italicus		E.carpathicus		B. xambeui
Sampling site		Fornace	Rancate	Monte	Sotpunt	San Carlo	Sta Maria	Balazuc	Lauris	Coglio	Vico Morcote	Mathis	La Morra	Amélie-les-Bains
(Sample size)		(10)	(15)	(11)	(13)	(12)	(12)	(24)	(22)	(10)	(10)	(22)	(12)	(6)
Locus	Allele													
AAT-1	123											1.00		1.00
	111												1.00	
	107							0.85	1.00					
	100													
	96	1.00	1.00	1.00	1.00	1.00				1.00	1.00			
	90							0.15						
	88													
	78						1.00							
AAT-2	117									1.00	1.00	0.93	1.00	
	113	1.00	1.00	1.00	1.00	1.00						0.07		
	110													
	107						1.00							
	100							1.00	0.95					
	96													1.00
	88								0.05					
ALPDH	109													1.00
	105												0.04	
	100						1.00	1.00	1.00	1.00	1.00	1.00	0.96	
	95	1.00	1.00	1.00	1.00	1.00								
ARK	104	0.05												
	100	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00			1.00	0.96	
	93									1.00	1.00		0.04	1.00
DDH	?													1.00
	102									1.00	1.00			
	101	1.00	1.00	1.00	1.00	1.00	1.00					1.00	1.00	
	100							1.00	1.00					
GAPDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	95													1.00
GTDH	104													
	100							1.00	1.00	1.00	1.00	1.00	1.00	
	90	1.00	1.00	1.00	1.00	1.00	1.00							
	50													1.00
HK	107													1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
	93									1.00	1.00	1.00	1.00	
IDH-1	100							0.75	0.88					
	95							0.25	0.12					
	94	1.00	1.00	1.00	1.00	1.00	1.00							
	93											0.32		
	90									1.00	1.00	0.68	1.00	
	77													1.00
IDH-2	100							1.00	1.00					
	93	1.00	1.00	1.00	1.00	1.00				1.00	1.00	1.00		
	89												1.00	1.00
	87						1.00							
MDH-1	113													0.50
	104													0.50
	100						1.00	1.00	1.00			0.05		
	89	1.00	1.00	1.00	1.00	1.00				1.00	1.00	0.95	1.00	
MDH-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
	89									1.00	1.00	0.95	1.00	
	77											0.05		1.00
MPI	135						1.00							1.00
	130	0.30	0.06											
	118	0.40	0.66			0.55								
	110				0.77	0.45								
	107	0.30	0.28	0.75										
	104												1.00	
	101									1.00	1.00			
	100			0.25	0.23			1.00	1.00					
	94											1.00		
PEP	107											1.00	0.33	
	104						1.00			1.00	1.00			
	100							1.00	0.89					1.00
	98	1.00	1.00	1.00	1.00	1.00							0.67	
	94								0.11					
6-PGD	110													1.00
	100							0.75	0.64					
	97	1.00	1.00	1.00	1.00	1.00	1.00					1.00	1.00	
	88							0.25	0.36					
	87									1.00	1.00			
PGI	105													1.00
	102							0.04						
	100							0.96	1.00	1.00	1.00			
	97											0.23	1.00	
	94				0.77	0.38								
	93	1.00	1.00	1.00	0.23	0.62	1.00					0.77		
PGM	100							1.00	1.00					
	98	0.20	0.43	0.27	0.15	0.25	0.33							
	96													1.00
	91	0.80	0.57	0.73	0.85	0.75	0.67							
	89											1.00		
	85												1.00	
	80									1.00	1.00			
PK	103									1.00	1.00	1.00	1.00	
	101	1.00	1.00	1.00	1.00	1.00								
	100							1.00	1.00					1.00
	98						1.00							
Mean no. of alleles / locus		1.2 (0.1)	1.2 (0.2)	1.1 (0.1)	1.2 (0.1)	1.2 (0.1)	1.1 (0.1)	1.3 (0.1)	1.2 (0.1)	1.0 (0.0)	1.0 (0.0)	1.3 (0.1)	1.3 (0.1)	1.1 (0.1)
Ho averaged (Directcount)		0.050 (0.031)	0.038 (0.028)	0.034 (0.024)	0.043 (0.030)	0.047 (0.024)	0.019 (0.019)	0.058 (0.029)	0.046 (0.024)	0.0 (0.000)	0.0 (0.000)	0.043 (0.022)	0.046 (0.037)	0.056 (0.056)
Percentage of loci polymorphic*		16.7	11.1	11.1	16.7	16.7	5.6	16.7	16.7	0.0	0.0	16.7	11.1	5.6

problematical in samples where $n < 10$. Departure from Hardy-Weinberg equilibrium was analysed for samples where $n \geq 10$.

Nei's genetic distance (1972) was calculated from pairwise comparisons of populations using the PHYLIP/GENDIST Phylogeny Inference Package, version 3.57c (Felsenstein, 1995). Using these distances as a matrix, a phenogram was created by average linkage cluster analysis (UPGMA) (PHYLIP/NEIGHBOR). Nei's distance measure is expected to rise linearly with time since separation of gene pools. The combination (Nei's genetic distance/UPGMA) is an efficient procedure for recovering an evolutionary tree (Nei *et al.,* 1985). Bootstrap values were obtained by 1000 pseudoreplicates (PHYLIP/ SEQBOOT) using *Buthus o. mardochei* from Agadir as outgroup.

Results

Allele frequencies and genetic variability

The allele frequencies of the twelve *Euscorpius* population samples and of *B. xambeui* are listed in Table 2 (allele frequencies of the Buthidae are not shown). In nearly all cases, genotype frequencies were consistent with Hardy-Weinberg expectations (HWE). Tests for HWE equilibrium were rejected at the 5% level in one out of eighteen cases in the populations of *E. germanus*. One out of eight tests deviated significantly in *E. carpathicus*. No significant deviations from HWE were obtained in *E. flavicaudis* (eight cases) and in *B. occitanus* (three cases). Sample sizes of $n < 10$ were not analysed.

We found that all populations are usually fixed for a certain allele at most gene loci analysed. The parameters of genetic variation are summarized for all Chactidae populations in Table 2. These parameters document the low level of genetic diversity within all samples. The genetic variability estimates of the Buthidae are not listed; however, they were comparable with those observed in the Chactidae. In samples where $n \geq 10$, the observed heterozygosity (H_o) ranged from 0.0 to 0.060 (mean 0.037 ± 0.024), the number of alleles per locus ranged from 1.0 to 1.3 (mean 1.17 ± 0.10), and the percent polymorphism ranged from 5.6 to 16.7 (mean 11.13 ± 6.42).

In contrast to the low variation within the scorpion populations, there was considerable variation among the populations with respect to the alleles observed. At eight out of eighteen loci scored, the *E. germanus* population from Sta Maria (Val Müstair) was fixed for alleles that were not found in the other samples of *E. germanus* (Table 2). The other five *E. germanus* populations were nearly identical, except for minor differences at polymorphic loci with respect to allele frequencies or rare alleles observed (e.g. loci PGM and MPI). Considerable variation among conspecific populations was also observed in *E. carpathicus*. The samples from Mathis and La Morra differed by allele substitution at four loci in addition to different allele frequencies at several other loci (Table 2). The comparison among species revealed that the *Euscorpius* species differed from one another at many gene loci.

Cluster analysis

Nei's genetic distance values were calculated in pairwise comparisons of all population samples (Buthidae included) and ranged from 0 to 2.890 D. A phenogram (Fig. 2) was derived from this distance matrix by UPGMA cluster analysis. Five populations of *E. germanus* clustered at a low level of genetic distance (0.003–0.024 D). The population from Sta Maria (Val Müstair), however, was separated and clustered with these at a distance of 0.307 D. Very low genetic distance was observed in the two *E. italicus* populations and the two *E. flavicaudis* populations, respectively. In contrast, the two *E. carpathicus* populations had a genetic distance of 0.177 D, while the distance found for the two *Buthus* subspecies was 0.063 D.

The branching point of the Sta Maria population and the other five *E. germanus* populations

Table 2: The observed allele frequencies of eighteen scored enzyme loci and the genetic variability estimates of thirteen Chactidae populations (SE in parentheses). Alleles are labelled by their relative mobility (for further details see Material and Methods).
*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

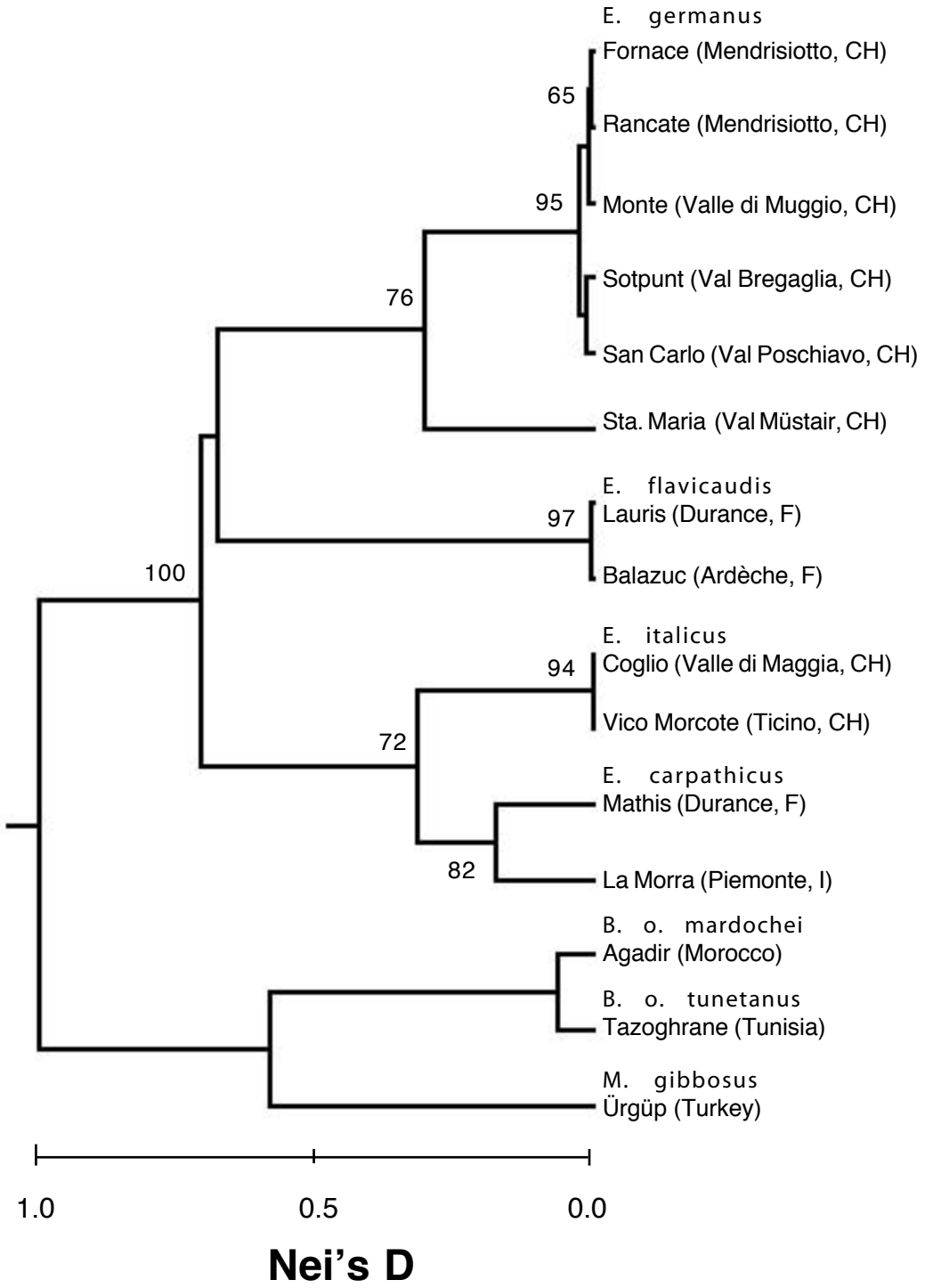

Fig. 2: Phenogram relating the population samples according to Nei's genetic distance. Numbers refer to bootstrap values (given as percentage over 1000 replicates) which were computed by resampling loci. The *B. occitanus* population from Agadir was used as outgroup.

was found nearly at the same distance level as the interspecific cluster of *E. carpathicus* and *E. italicus*, whereas the two *B. occitanus* subspecies clearly clustered at a lower distance. *B. occitanus* and *M. gibbosus* clustered at a distance of 0.587 D, whereas the branching point of the *Euscorpius* species was clearly at a higher distance (0.713 D). *B. xambeui* could not be included in the cluster analysis because it showed $D = \infty$ if compared with the *E. germanus* samples.

Discussion

Allozyme data

The allozyme data indicated a low level of genetic diversity within populations (mean observed heterozygosity 0.037 ± 0.024, mean percentage of polymorphic loci 11.13 ± 6.42, mean number of alleles 1.17 ± 0.1, over 13 populations $n \geq 10$). In contrast, Yamashita & Polis (1995b) found higher levels (mean heterozygosity 0.143, mean percentage of polymorphic loci 53.13, mean number of alleles 1.65) in the sand scorpion *Paruroctonus mesaensis* (Vaejovidae). However, these two data sets are difficult to compare because different sets of enzymes have been analysed. Furthermore, Yamashita & Polis's mean is based on eight loci, whereas 18 loci were used in the present study. The low level of polymorphism in *Euscorpius* may be caused by a low mutation rate, by natural selection or by a high degree of inbreeding possibly due to bottlenecks (Avise, 1994), while a low mutation rate would need efficient repair mechanisms. At present, there is no information as to which of these factors is more likely to account for the low polymorphism observed.

Cluster analysis

The Sta Maria population was highly differentiated from the other *E. germanus* populations, with a distance of 0.307 D in the cluster analysis. With respect to conspecific populations, a rather high distance was also observed between the two *E. carpathicus* populations, whereas the two *E. flavicaudis* and *E. italicus* populations showed very moderate or no differentiation, respectively. The geographical distance between the two *E. carpathicus* populations is much larger than the geographical distances between the sampling sites of the other three *Euscorpius* species. Moreover, the *E. carpathicus* populations are separated by topographical barriers. This is, however, not the case in the *E. germanus* populations and may therefore be taken as an indication that the Val Müstair scorpions belong to a population group that was separated from the western population group long ago and has diverged since that time. This speculation is supported by data from a few *E. germanus* specimens from the Alto Adige (Völs and Gummer) and from the Trentino (Vetriolo). These scorpions show the same alleles at the eight diagnostic gene loci as the sample from Sta Maria. Our molecular data support the morphological distinction of *Euscorpius g. germanus* (Alto Adige, Trentino, Cadore) and *Euscorpius g. alpha* (Alpi Lombarde, Ticino, Valtellina, Grison) (Capra, 1939; di Caporiacco, 1950; Bonacina, 1980).

It is of interest that the level of differentiation which we recorded for the *E. germanus* populations considerably exceeds the level that is found for the two *Buthus* subspecies. These comparative data suggest that the southern Swiss valleys must have been colonized from separate refuges after the last glaciation. Speculating about the possible age of the two *E. germanus* population groups, we refer to the molecular clock literature, where estimates of divergence time t from genetic distance D are related by the equation $t = kD$ (Nei, 1987b). Estimates for the parameter k range from 8.1×10^5 to 18×10^6 (Nei, 1987b); however, the compilation covers vertebrate species exclusively. A recent study on pairs of marine sister taxa of the snapping shrimp *Alpheus* across the Isthmus of Panama (Knowlton *et al.*, 1993) revealed a rate of protein divergence of 0.03 to 0.04 Nei's D per 10^6 years. If the divergence rate of these shrimps is applied to *E. germanus*, we end up with a time of about 7.5 M to 10 M yrs BP for the separation of the two population groups. This appears unrealistic, but, it supports our view that the *E. germanus* population groups are unexpectedly highly differentiated. It is also interesting that the level of genetic differentiation that we found in the genus *Euscorpius* exceeds the differentiation between *B. occitanus* and *M. gibbosus*. This suggests that the genera *Buthus* and *Mesobuthus* diverged more recently than the species of *Euscorpius*.

Acknowledgements

We thank Victor Fet for critical comments on the manuscript and Mathias Lörtscher for assistance in statistical analysis.

References

AVISE, J. C. 1994: *Molecular markers, natural history and evolution*. New York: Chapman & Hall.

BONACINA, A. 1980: Sistematica specifica e sottospecifica del complesso *"Euscorpius germanus"* (Scorpiones: Chactidae). *Riv. Mus. civ. Sci. nat. Caffi* **2**: 47–100.

BRAUNWALDER, M. E. & TSCHUDIN, M. 1997: Skorpione. Eine Einführung mit besonderem Augenmerk auf beide Schweizer Arten. *Biol. einheim. Wildtiere* **1**/47 (Infodienst Wildbiologie und Ökologie, Zürich).

CAPORIACCO, L. di 1950: Le specie e sottospecie del genere „*Euscorpius*" viventi in Italia ed in alcune zone confinanti. *Atti Accad. naz. Lincei Memorie* (*ser. 8*) **2**: 159–230.

CAPRA, F. 1939: L'*Euscorpius germanus* (C. L. Koch) in Italia (Arachn. Scorpiones). *Memorie Soc. ent. ital.* **18:** 199–213.

ĆURČIĆ, B. P. M. 1972: Considerations upon the geographic distribution and origin of some populations in the genus *Euscorpius* Thorell (Chactidae, Scorpiones). *Rapp. Comm. int. Mer Medit.* **21** (3): 83–88.

FELSENSTEIN, J. 1995: PHYLIP (Phylogeny Inference Package), Version 3.57c. Seattle: University of Washington.

FERGUSON, A. 1980: *Biochemical systematics and evolution*. New York: Blackie & Son Ltd.

FET, V. 1993: Notes on *Euscorpius mingrelicus* (Kessler, 1874) (Scorpiones: Chactidae) from the Caucasus. *Riv. Mus. civ. Sci. nat. Caffi* **16**: 1–8.

FET, V. & BRAUNWALDER, M. E. 1997: On the true authorship and taxonomic history of *Euscorpius germanus* (C. L. Koch, 1837) (*nec* C. L. Koch, 1836; *nec* Schaeffer, 1766) (Scorpiones: Chactidae). *Bull. Br. arachnol. Soc.* **10**: 308–310.

FUTUYMA, D. J. 1986: *Evolutionary biology*. Sunderland, MA: Sinauer Associates.

HADZI, J. 1931: Der Artbildungsprozess in der Gattung *Euscorpius* Thor. *Archo zool. ital.* **16**: 356–362.

HARRIS, H. & HOPKINSON, D. A. 1976: *Handbook of enzyme electrophoresis in human genetics*. Amsterdam: North Holland.

HARTL, D. L. 1988: *A primer of population genetics*. Sunderland, MA: Sinauer Associates.

KINZELBACH, R. 1975: Die Skorpione der Ägäis. Beiträge zur Systematik, Phylogenie und Biogeographie. *Zool. Jb.* (*Syst.*) **102**: 12–50.

KNOWLTON, N., WEIGHT, L. A., SOLORZANO, L. A., MILLS, D. K. & BERMINGHAM, E. 1993: Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science, N.Y.* **260**: 1629–1632.

MURPHY, R. W., SITES, J. W., BUTH, D. G. & HAUFLER, C. H. 1996: Proteins: Isozyme electrophoresis. *In* D. M. Hillis, C. Moritz & B. K. Mable (eds.). *Molecular systematics*. Sunderland, MA: Sinauer Associates: 51–120.

NEI, M. 1972: Genetic distance between populations. *Am. Nat.* **106**: 283–292.

NEI, M. 1987a: *Molecular evolutionary genetics*. New York: Columbia University Press.

NEI, M. 1987b: Genetic distance and molecular phylogeny. *In* N. Ryman & F. Utter (eds.). *Population genetics & fishery management*. Seattle: University of Washington Press: 192–215.

NEI, M., STEPHENS, J. C. & SAITOU, N. 1985: Methods for computing the standard error of branching points in an evolutionary tree and their application to molecular data from humans and apes. *Molec. Biol. Evol.* **2**: 66–85.

PASTEUR, N., PASTEUR, G., BONHOMME, F., CATALAN, J. & BRITTON-DAVID, J. 1988: *Isozyme genetics*. University of Montpellier: Ellis Horwood Ltd.

POLIS, G.A. 1990: Ecology. *In* G. A. Polis (ed.). *The biology of scorpions*. Stanford, CA: Stanford University Press: 247–294.

SCHOLL A., CORZILLIUS, B. & VILLWOCK, W. 1978: Beitrag zur Verwandtschaftsanalyse altweltlicher Zahnkarpfen der Tribus Aphaniini (Pisces, Cyprinodontidae) mit Hilfe elektrophoretischer Untersuchungsmethoden. *Z. Zool. Syst. EvolForsch.* **6**: 116–132.

SWOFFORD, D. L. & SELANDER, R. B. 1989: *BIOSYS–1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics*. Release 1.7. Urbana: University of Illinois Press.

VACHON, M. 1962: Remarques sur l'utilisation en systématique, des soies sensorielles (Trichobothries) chez les scorpions du genre *Euscorpius* Thorell (Chactidae). *Bull. Mus. natn. Hist. nat. Paris, sér. 2* **34**: 345–354.

YAMASHITA, T. & POLIS, G. A. 1995a: A test of the central-marginal model using sand scorpion populations (*Paruroctonus mesaensis*, Vaejovidae). *J. Arachnol.* **23**: 60–64.

YAMASHITA, T. & POLIS, G. A. 1995b: Geographical analysis of scorpion habitat islands. *Heredity, Lond.* **75**: 495–505.