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Genetic variability and incidence of systemic diseases in wild vines (*Vitis vinifera ssp. silvestris*) along the Danube

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Summary

In the riparian woods of Danube and March east of Vienna 87 wild specimens of *Vitis vinifera ssp. silvestris* were genetically analysed and compared. The *silvestris* population can be split into 6 distinct groups, but this clustering cannot be explained solely by the geographical distance. The unique genetic variability observed represents a strong case for preservation of wild grapevines.

The incidence of bacterioses, viroses and nematodes transmitting nepoviruses to these vines were registered. None of the analysed specimens suffered from *Agrobacterium vitis*-induced crown gall. Only some vines were infected by viral pathogens such as GLRaV I and SLRV. Thus the wild vines do not constitute a risk for the surrounding commercial vineyards. On the other hand, diseases spread from cultivated grapevines may seriously harm the wild vine population.

Four species of nematodes transmitting nepoviruses were registered. Samples of *Xiphinema vuittenezi* and *Longidorus attenuatus* from the Lobau (natural forests, north of the Danube in the area of Vienna) differ morphometrically from others found on arable soils or isolated from the research area.

K e y w o r d s : microsatellites, grapevine, nematodes, nepoviruses.

Introduction

On the river plains east of Vienna the wild grapevine *Vitis vinifera* ssp. *silvestris* is native (Jacquin 1762, Kirchheimer 1955) and originally was very abundant. This seems to have changed in the 20th century since Kirchheimer (1955) recognised only 20 specimens in the Lobau (area of Vienna) and 25 near Orth (location at the Danube), some isolated ones were found on both banks of the river Danube between Vienna and the Austrian-Slovakian border. Kirchheimer (1955) was quite sure, that the native vines were extinct in the floodplains of the March river. In the next 40 years, there was little interest concerning wild grapes in Austria. After the "Nationalpark Donauauen" and protection areas of the World Wildlife Fund (WWF) near Regelsbrunn/Danube and Marchegg/March were founded, wild grapevines of Austria are of interest for conservation and scientific research.

In 1996 the WWF started a cultivation/recultivation programme for wild grapes, with the aim to reduce their possible extinction in Austria. Arnold *et al.* (1998) investigated the occurrence of wild vines in Europe and listed some specimens for Marchegg near Orth/Danube and in the Lobau near Vienna. Recently C. Freiding and C. Gussmark, with the support of C. Fraissl, began to map wild vines within the national park Donauauen. They found about 180 individual vines.

Our research activity started in 2001. The first intention was to investigate the number of remaining individuals and to map individuals. The main interest was focused on the genetic variability of wild grapes and their diseases, mainly the incidence of viroses and bacterioses. We also evaluated the possible occurrence of transmission from the cultivated grapevine of the nearby vinegrowing regions or *vice versa*. The presence of viral pathogens and their vectors was investigated. Especially nepoviruses that are easily transferred by nematodes constitute a menace for wild grapevines.

Material and Methods

The geographical position of each analysed wild grapevine was registered using the Global Positioning System (GPS), therefore anybody has the opportunity to follow and extend the outcome of this study. Sampling regions are shown in Fig. 1. Samples for genetic comparison were taken from the shoots.The genetic profile of wild grapes was gained by

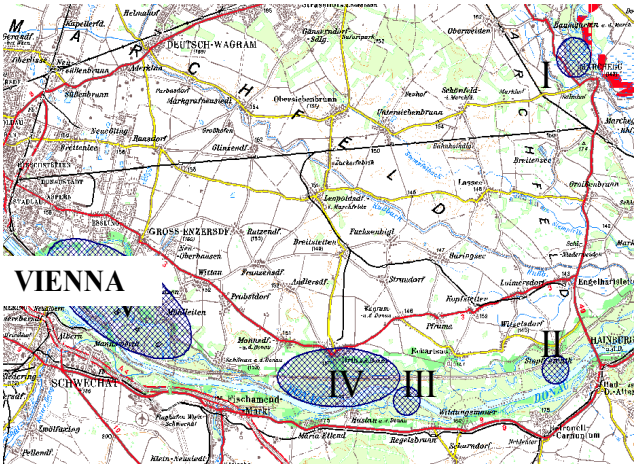

Fig. 1: Occurrence of wild vines of *Vitis vinifera* east of Vienna. Sampling areas I to V.

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genotyping all individuals with 18 SSR markers. DNA was extracted from young leaves of naturally grown shoots by following the protocol of THOMAS *et al*. (1993) modified by REGNER *et al*. (1998). PCR conditions were applied as used for identification of grapevines (REGNER *et al.* 2000). The amplified allelic fragments were separated on a 6 % polyacrylamide gel on a H373 Sequencer (Applied Biosystems). The fluorochrome-labelled primers (Fam, Tet, Hex) allowed an automatic estimation of the length by using GenScan 350 Tamra as an internal standard. The following SSR loci are involved in the study: The VVS2 marker was developed by THOMAS and SCOTT (1993) and the VVMD 5, 6, 7, 8, 24, 25, 27, 28, 36 markers by Bowers *et al*. (1996) as well as by BOWERS and MEREDITH (1999). The VRZAG 7, 12, 15, 21, 62, 67, 79 markers (SEFC *et al*. 1999) were obtained from investigations into simple sequence repeats of *Vitis riparia*. Only the VMC 62 marker is not from the public domain as this marker is still not published and is part of the *Vitis* Microsatellite Consortium database (managed by Agrogene, F). The data were analysed by using the Microsat program and the multivariate comparison was drawn with the PhyQuest program (TIEFENBRUNNER *et al.* 2002) .

Samples from roots, tendrils and shoots of vines were taken to identify viral and bacterial (*Agrobacterium vitis*) pathogens. DAS-Elisa tests were done for the following viruses: Grapevine Fanleaf virus (GFLV), Arabis Mosaic virus (ArMV), Raspberry Ringspot virus (RpRSV „ch“ and „g“), Strawberry Latent Ringspot virus (SLRSV), Tomato Ringspot virus (ToRSV „ch“ and „pybm“), Alfalfa mosaic virus (AMV), Tobacco Ringspot virus (TRSV), Grapevine Fleck virus (GFkV), Grapevine Virus A (GVA) and Grapevine Leafroll associated virus I, III and VI (GLRaV I, III, VI). For identification of *Agrobacterium vitis* the method of DNA isolation and PCR described by SCHULZ *et al*. (1993) was used, except that no additional extension time was applied. The primers acs1, acs2 and vis1, vis2 were purchased from VITOLAB (T. F. Schulz, Bahnhofstr. 46, 74348 Lauffen a. N., Germany).

For the recording of soilborne virus vectors soil samples mainly from the rhizosphere of the wild vines were taken with a cylindrical soil auger (22 mm diameter). Samples were taken down to 90 cm, they had a volume of ca. 340 cm^3. For the extraction an Oostenbrink-Elutriator was used (sampling sieve width: 150 mm). All nematodes were extracted and those from the family *Longidoridae* were identified at the species level. For identification the following polytomous keys were used: Genus *Longidorus* - CHEN *et al*. (1997 and Suppl. 1), LOOF and CHEN (1999); *Xiphinema* with exclusion of *X. americanum s. l.* - LOOF and LUC (1990 and Suppl. 1) , LOOF and LUC (1993).

The programme PhyQuest was used for a multivariate comparison of biometrical data (the method is described in TIEFENBRUNNER *et al.* 2002). Species and local populations of the nematode family *Longidoridae* were compared. Within the genus *Longidorus* characters like body length, body diameter at vulva, tail length, body diameter at anus, distance oral aperture to vulva, odontostyle, distance oral aperture to guiding ring, body diameter at guiding ring and body diameter at lip region were applied. For identification of *Xiphinema* the same characters as for *Longidorus* and additionally the length of the odontophore were applied. The map (Fig. 1) was produced with the aid of the program Austrian MAP (data from Bundesamt für Eich- und Vermessungswesen, software from DaimlerChrysler Aerospace).

Results and Discussion

Description of the sampling sites: We chose 5 sampling areas (Fig. 1): Area I: Marchau, WWF protection area, north of Marchegg, Lower Austria; Area II: Stopfenreuth, Nationalpark Donauauen, Lower Austria; Area III: Regelsbrunn, WWF protection area, Lower Austria; Area IV: Orth/Danube, Nationalpark Donauauen, Lower Austria; Area V: Lobau, part of the Nationalpark Donauauen, east of Vienna.

Area I: Surrounds the riparian woods of the March north of Marchegg close to the Slovakian border. Thirty one samples of wild grapevine individuals were taken. Most sites were on the banks of river branches. The wild vines were clustered in groups. Most individuals lacked inflorescences and therefore the sex could not be determined. Two were definitively females and 6 were male. The most common host plants of this liana were *Quercus* species (Tab. 1). More information about the sampling sites from this area is available from TIEFENBRUNNER *et al.* (2004 a).

Area II: Some soil samples (merely to close a relatively large geographical gap) were taken but this region lacked wild vines.

Area III: The southern bank of the Danube near Regelsbrunn, was found to host only a few wild grapevines. They all had male flowers and all grew on *Populus* hybrids close to the river.

Area IV: The floodplain north of the Danube near Orth/Danube. Samples of 23 *Vitis* individuals were taken. 11 were female, 9 male, one hermaphrodite and 2 with unknown sex. They were relatively homogenously distributed. The *Vitis* hosts *Cornus sanguinea* and *Crataegus monogyna* dominated. Many sampling sites were located far away from the river or its branches, and thus the soil was drier. More details about this area can be derived from TIEFENBRUNNER *et al*. (2004 a).

Area V: The riparian woods (Lobau) of the Danube near Vienna. Twenty nine vine samples were taken. As in area IV, *Cornus sanguinea* and *Crataegus monogyna* were very common hosts, as well as *Populus* species (Tab. 1). Many vines had a distance of more than 100 m to a river branch. Due to late sampling the sex was not determined.

Overall, this study contains data from 87 *Vitis ssp. silvestris* genotypes. Vines from the WWF protected regions (areas I and III) were not registered by C. Freiding and C. Gußmark. Therefore 35 wild vines of the Austrian riparian woods can now be added to the Freiding/Gußmark map. In area V this map was helpful but also other vines were discovered. Finally the total number of definitely known specimens was raised to at least 220.

Genetic analysis: One of the main tasks was to ask whether the populations of area I, III, IV and V were homozygous. Using 18 different SSR (simple sequence repeats) loci a general genetic profile determined them as

T a b l e 1

Number of *Vitis* hosts in different areas (hosts, that are very frequent in an area are accentuated)

Hosts	March Area I	Danube Area III & IV	Danube Area V
Acer campestre	9	7	4
Alnus glutinosa	1	5	0
Betula pendula	0	0	1
Carpinus betulus	6	1	0
Corylus avellana	2	2	0
Cornus mas	0	3	0
Cornus sanguinea	2	16	11
Clemathis vitalba	0	1	4
Crataegus monogyna	5	14	13
Euonymus vernalis	0	0	1
Fraxinus excelsior	6	7	3
Ailanthus altissima	0	1	0
Rhamnus frangula	0	1	0
Humulus lupulus	0	1	0
Ligustrum vulgare	0	1	5
Pyrus pyraster	0	1	0
Populus spp.	7	7	13
Prunus spinosa	0	1	0
Quercus spp.	14	2	2
Robinia pseudacacia	0	1	0
Salix spp.	1	4	3
Sambucus nigra	0	2	4
Ulmus laevis	5	0	3
Viburnum opulus	0	1	0
Number of *Vitis* specimens	31	27	29

silvestris genotypes (Tab. 2). No individual showed a close relationship to the cultivated grapevines examined (Tab.3). Therefore these individuals may represent unique germplasm. For comparing their genetic relationship we conducted a multivariate comparison of the distance of all specimens from one area. According to this, area I and III are homogenous, whereas IV and V, are clearly split into two distinct genetic groups. In both cases most individuals belong to one group, whereas only few (4 or 2) belong to the other. Nei's genetic distance for populations (Nei 1972) was applied to compare all groups (Fig. 2). The results indicated that there is no correlation between the geographical and the genetic distance. For instance genotypes of area I and the larger group of area V show highest similarity. The individuals on area V grow on sites with the largest geographical distance. The larger group of area IV is genetically less similar to the second group of this area than to the vines of area I or to the larger group of area V.

Specimens of area III and from the smaller group of the area V are distantly related, but completely different from all the others. These two groups are also morphologically very different, concerning leaf shape, vigour and fruit ripening.

The unexpected pattern of genetic and geographical divergence is not in accordance with the spread of natural populations. The smaller and genetic very divergent groups were possibly carried as seeds in the gut of birds to distant places, or more likely survived. Due to the historical records of the abundance of wild vines we suppose that only a few of the autochthonous vines survived. If wild vines today are only a relic of large populations of the past, the further extinction of only a small group of specimens would lead to an important loss of genetic variability. This seems to be especially true for the smaller group of the Lobau. According to Kirchheimer (1955) near Vienna wine of acceptable quality was produced from native wild vines until 1911.

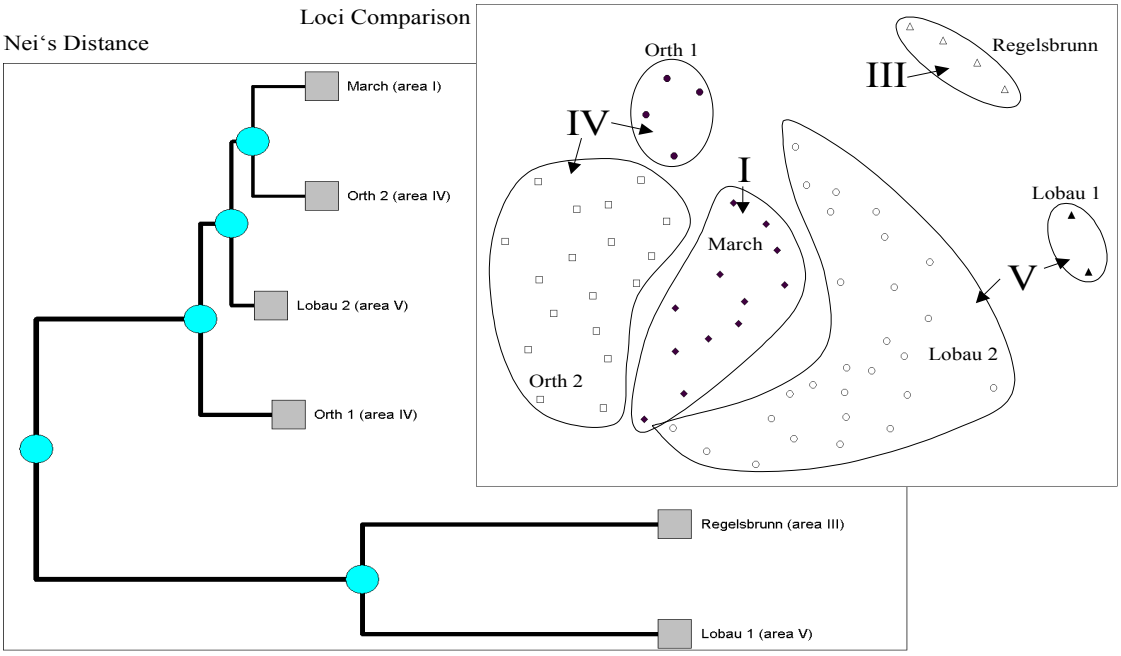

Fig. 2: Multivariate comparison of the genetic relationship of the specimens (right) and groups (left). The latter are compared using Nei's (1972) genetic distance of populations.

Table 2

Alleles of analysed wild wines - examples of group

Vines	VVS2	MD5	MD7	MD27	VRZag62	VRZag79	MD 6	MD 8	MD 24
L 5	132/151	231	240	188	195	252	199/206	139	212
L 6	132	231	240/264	189	198	239/252	199	139	212/216
L 7	132/159	233/265	240/254	189/217	195	246/258	205/210	141/175	206/216
L 8	132/159	234/266	240/254	189/217	188/195	146/258	204/209	142/175	206/226
L 9	131/151	231	240/264	189	195	239/252	199	139	212
L 10	136/143	231	240/264	189	195	252	199	139	212
L 11	131	231	240/264	189	195	252	199/206	139	212/216
L 12	151	231	242/264	189	195	252	200/207	139	212/216
L 13	151	231	264	189	195	252	199	139	212/216
L 14	151	227/231	240	189	185/195	252/256	199/206	139	212
L 21	132	227	240/264	189	195	248/256	199/206	139	212/216
L 22	132/151	232	239/263	188	195	246/252	199	139	212
L 23	132	232	239	188	195/197	252/256	199	139	212
L 24	151	228/232	241/249	188	195/197	246/252	199	139	212/216
L 25	132	232	239/249	188	195/197	246/252	199	139	212/216
R 1	137/141	252/264	253	197/215	189/191	256/262	204/214	175/177	206
R 2	137/141	252/264	253	197/215	189/191	256/262	204/214	175/177	206
R 3	137/141	252/264	253	197/215	189/191	256/262	199/204/14	175/177	206
Orth 1	128/133	226/236	239/263	189	195	252	200/207	139	195/213
Orth 2/0	128/133	228/232	241	189	195	252	207	139	217
Orth 3/0	133/152	228/232	241/265	192	195	252	184/200	139/141	213/217
Orth 4	146/152	232	241	190/1	195	252/256	200/207	139/141	213/217
Orth 5	128/133	232	239/263	189	195	252	207	140	217
Orth 6	133/152	232	243/265	189	195	252	207	139	195/213
Orth 8	128/133	232/240	241	192	195/197	252	200/207	142	213/217
Orth 9	133/146	232/266	239/253	189/211	193/195	256/260	214	139	207/217
Orth 11	133/152	232	240/264	191	195	252	200	139	213/217
Orth 14	142/153	232	263	189	195	252	207	140	195/213
Orth 15	133/142	232	259/264	189	195	246/252	200/207	139/40	213/219
Orth 16	128/152	232	241	192	195	252	200/207	139	195/213
Orth 17	133/152	232	239/40	189/191	195/197	252/256	184/200	139	198/217
Orth 18	133/152	232/238	241	189/191	195	252	199/206	139	213/217
M 1	132/152	228	240/258	189	194/198		199	139	213/217
M 6	152	228/232	240/258	181/189	196	244/252	199/208	139	213
M 10	132/152		240	189/191	193/196	252/256	199	139	213/217
M 12	139	228/232	240/264	189	195	252	206	139	213/217
M 13	132	228	240	189	195	252	199/206	139	213
M 17	132	228	250/264	189	193/195	252	199	139	213
M 20	152	228/232	240/251	189	195	252	199/207	139	213/217
M 24	132	232	256/264	189	193/195	252	199/206	139	213/217
M 25	132	228/240	264	191	193/195	252/256	199/206	139	213
M 27	132	228/232	240/242	189	196	252	199	139	213/217
M 28	132	228/232?	240/258	125	193/196	252	199/206	139	213

Nowadays only two vines of this group produce grapes that could be used for wine production.

The larger groups are closer related. This may indicate that the genetic background of these vines favour their occurrence under the specific climate and growing conditions along the Danube. Thus the size of the smaller groups could be reduced due to their limited survival as a result of lower genetic adaptation to the prevailing environmental conditions.

The specimens of the closer areas IV and V are more distantly related than to the geographically more distant area I. A further argument for the relic theory is the knowledge that no spreading barrier was between the regions of the Lobau (area V) and of Orth (area IV) in the last millennia.

Viruses and bacterioses: *Agrobacterium vitis* could not be recorded in any of the analysed areas. However, viruses were found in area IV, 6 specimens were GLRaV I positive and one SLRV was detected (Tab. 3). No evidence

Tab. 2, continued

Vines	MD 25	MD 28	MD 36	VMC 62	VRZag7	VRZag 12	VRZag 15	VRZag 21	VRZag 67
L 5	257/268	237	254/295	224	157	155	181	192	153
L 6	257/268	238/266	295	228	157	155	177/201	192/196	153
L 7	241/268	220/238	234/41/48/54	213/224	157/192	142	166	202/206	139/157
L 8	241/268	220/238	240/254	213/224	157/192	141	166	201/205	139/157
L 9	268	238/266	254/295	224/228	109/157	155	179/181	192/196	153
L 10	268	238/266	254/295	224/228	109/157	155	181	192/195	153
L 11	256/268	238/266	295	224/228	157	155	181/189	192/196	132
L 12	251/257	238	295	209/224	157	155	177/181	192/196	153
L 13	257	238	295	209/224	157	155	181/201	192	132/153
L 14	268	237	295	224/228	157	155	185/201	192	153
L 21	257	237/265	254/294	223/228	109/156	155	181/188	186/192/197	153
L 22	269	237/265	294	223	156	155	199/201	191/195	153
L 23	256/270	(254)/265	295	223/228	156	155	182/201	191/195	153
L 24	251/257	237/265	254/295	223/228	156	155	189/201	191/195	153
L 25	257/268	263/265	295	228	156	155	181/201	191/196/201	132/153
R 1	239/241	235	240	213	178/187	142/171	164/166	199/206	157/167
R 2	239/241	235	240	213	178/187	142/171	164/166	199/206	157/164
R 3	239/241	235	240	213	178/187	142/171	164/166	199/206	157/164
Orth 1	256	236	254/295	224	109/156	155	201	192	152
Orth 2/0	256/268	238/266	255/295	224	156	155	181/201	196	153
Orth 3/0	247/257	238/266	295	224/232	156	155	181/203	192/204	153
Orth 4	256	254/266	254/295	224/241	156	155	181	196	153
Orth 5	256/268	237/265	295	224	156	155	177/181	192	151/153
Orth 6	256	238	254/295	210/228	156	155	181/201	192	152/155
Orth 8	256	238/242	295	224/228	109	155	181	196	151/153
Orth 9	240/256	218/266	241/295	210/214	156	141/157	177/185	196	153
Orth 11	250/268	266	295	224/228	156	155	177	192/196	153
Orth 14	250/256	238/266	295	225/229	156	155	189/201	192/196	153/155
Orth 15	251/257	238/266	254/295	224/228	156	155	181/201	192/196	153/155
Orth 16	250/256		295	255/259	156	155	177/181	192/196	153
Orth 17	250/256	235/238	245/295	210/224	156	155	181/201	192/196	153
Orth 18	256/268	238/166	245/295	224	156	155	199	194	153
M 1	257/269	265	295	220/224	156	155	181/201	196	132/153
M 6	268	237/265	254/295	220/224	109/156	155	177/203	192	153
M 10	257/269	265		220/224	156	155	178	192/196	130/153
M 12	251/269	237/265	254	220/224	156	155	177/181	192/196	153
M 13	257	237/265	295	220/224	156	156	177	192/196	153
M 17	251/269	265	295	220/224	109/156	155	178	194/196	153
M 20	257	265	270/295	220/224	109/156	155	178	192/196	153
M 24	257/268	237/265	295	220/224	156	155	177	192/196	132/153
M 25	268	237	295	224/228	156	155	181/203	192/196	132/153
M 27	251/257	265	254	220/224	156	155	177/189	192/196	153
M 28	257		254	224/228	109/156	155	177/189	192/196	153

for virus-induced pathogens was found in the other areas. We took samples from roots, wood, tendrils, shoot tips, shoots, inflorescences, leaves and petioles). In the case of GLRaV I, a molecular biological proof was only possible from inflorescences, in the case of SLRSV from roots. It appeared, that only parts of the large vines suffered from a disease but not the whole plant.

Area III and IV are bordering the vine growing region Carnuntum. In commercial vineyards several grapevine viruses and *Agrobacterium vitis* were found (Gangl *et al*. 2001). Despite this no viruses and *Agrobacterium vitis* were detected in wild vines. Thus there is no risk for the economically grown grapevines from the wild vines of the floodplains. On the other hand, the future of these wild vines will only not be endangered if plantings are free of viruses and bacteria. In the long term the existence of wild vines depends of the usage of certified grapevine material to reduce the risk of pathogen spread.

Nematode vectors of viruses and other longidorids: Four nepovirus vector species (Brown and Trudgill 1997) were registered in the riparian woods of Danube and March. *Longidorus attenuatus*, the vector of

Table 3

SSR alleles of autochtonous Mid Europe cultivars

Cultivar	VVS2		MD 5		MD 7		MD 27		VRZAG 62		VRZAG 79	
Blauburger	143	151	232	240	243	249	179	181	189	205	237	259
Blaufränkisch	143		226	240	239	249	179	194	195	205	237	251
Bouvier	133	151	228		243		185	194	195	197	239	251
Goldburger	135	143	238		239	247	189		195	197	251	259
Gutedel B	133	143	228	236	239	247	185	189	195	205	251	259
Jubiläumsrebe	151		232	240	243	247	181	189	189	205	251	259
Müller Thurgau	143	151	226	228	247	257	181		195		243	245
Muskateller B	133		228	236	233	249	179	194	189	195	251	255
Muskat Ottonel	133	143	226	228	239	243	179	189	189	195	255	259
Neuburger	131	151	226	240	247	253	189	194	193	205	251	
Orangetraube	137	143	230	236	239	247	189		195	205	245	259
Portugieser Bl.	143	151	226	232	243	255	181	194	189	205	249	259
Rheinriesling	143	151	226	234	249	257	181	189	195	205	243	245
Rotgipfler	133	151	232	246	239	257	183	189	189	197	251	
Sauvignon Bl.	133	151	228	232	239	257	175	189	189	195	245	247
Scheurebe	143	151	226	238	247	249	189	194	189	205	245	249
Silvaner B	151	153	226	232	243	247	189	194	189	205	249	251
St. Laurent	137	151	228		239	257	185	189	195		239	247
Traminer RG	151		232	238	243	257	189		189	195	245	251
Veltliner Rot	131	133	240	246	239	253	183	194	193	197	251	
Veltliner FR.	133	151	232	240	247	253	189	194	193	205	251	
Veltliner Grün	133	151	232		247	257	189	194	195	205	245	249
Welschriesling	135	151	226	238	247	257	185	189	195	197	251	
Wildbacher Bl.	143	151	228	240	239		181	191	195	197	243	251
Zierfandler	133		240		243	253	189	194	189	193	245	251
Zweigelt	137	143	226	228	239		179	185	195		237	239

the Tomato black ring virus, *L. elongatus*, vector of Tomato black ring and Raspberry ringspot virus, *L. macrosoma*, vector of the Raspberry ringspot virus and *Xiphinema diversicaudatum*, vector for the transmission of the Arabis mosaic and the Strawberry latent ringspot virus (Tab. 4) were found. Most soil samples were taken from the rhizosphere of *Vitis* but additionally some samples were taken from meadows and forest soils. The only species strictly associated with wild grapes seems to be *L. elongatus*.

Area I is dominated by *L. intermedius*. This species is known to be ecologically connected with oak trees that are very frequent in the floodplains of the March. *L. poessneckensis* and *X. diversicaudatum* are common near river branches. The number of samples in area II and III was too small to get a representative view. The incidence of *L. juvenilis* in area III (the only one in Austria) seems interesting.

Area IV has by far the most diverse longidorid fauna - 10 species, but none is very abundant, although *L. macrosoma* dominates slightly. In two samples we found *X. vuittenezi*, a species, that is common in vineyards, but seldom occurs in riparian woods. In area V, *L. attenuatus* is extremely common and can be found in 17 out of 29 samples. Even more abundant per sample is *X. vuittenezi*. We recognised it in 10 out of 29 samples. *X. vuittenezi* was detected only in area IV and V. Longidorids can be determined correctly by means of morphometric analysis. The results showed that *L. attenuatus* and *X. vuittenezi* of the riparian woods morphometrically differ from individuals of the same species isolated from vineyard locations.

Fig. 3 shows the result of a multivariate analysis of body proportions. Compared are *Xiphinema vuittenezi* 1) from vineyards and arable land, 2) from area IV (Orth/Danube), 3)

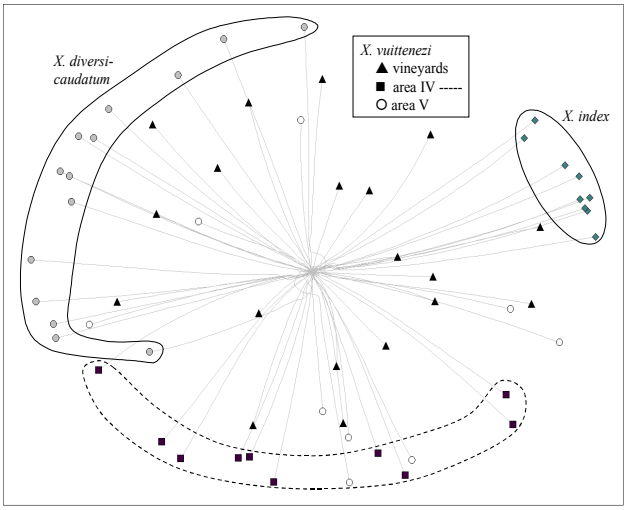

Fig. 3: Morphometrical comparison of three local 'populations' of *Xiphinemia vuittenezi*, *X. diversicaudatum* and *X. index* are used as outgroups.

T a b l e 4

Nematodes of soil samples from the floodplains of Danube and March

Nematodes Order	Genus or Family	Species	Area I	Area II	Area III	Area IV	Area V	Total
Dorylaimids	*Longidorus*	*L. attenuatus*	5	5	0	23	339	372
		L. elongatus	0	0	0	4	0	4
		L. intermedius	509	0	0	5	13	527
		L. juvenilis	0	0	1	0	0	1
		L. macrosoma	0	0	0	91	0	91
		L. poessneckensis	76	2	0	28	0	106
		L. sp.	0	0	0	7	0	7
		L. sp.	0	0	0	13	0	13
	Xiphinema	*X. diversicaudatum*	203	0	19	62	99	383
		X. pachtaicum	0	0	0	1	24	25
		X. vuittenezi	0	0	0	28	551	579
	Trichodorus	*T. sp.*	0	0	0	0	1	1
	others		496	0	18	440	416	1352
Rhabditids			39	0	1	49	44	132
Mononchids			152	0	1	28	4	184
Tylenchids	*Criconematidae*		14	0	0	160	6	180
	others		1	0	3	101	5	107
								4064
		Samples	31	2	4	32	29	

from area V (Lobau), and 4) *X. index* and 5) *X. diversicaudatum* as morphometrically similar, but nevertheless clearly distinguishable 'outgroups'. In Fig. 3 *X. index* and *X. diversicaudatum* are at the periphery and are well separable from the other groups. The specimen from area IV are also on the periphery, between the outgroups. The individuals of area IV are separable from the one originating from vineyards and arable land (in the centre of Fig. 3), thus indicating that they could belong to different 'species'. Indeed this was the original interpretation (Tiefenbrunner *et al*. 2004 a, b) before the animals of area V were known.

In the past within longidorid systematics, splitting was always preferred to lumping. Especially when agamotaxons are concerned it was not helpful. It is questionable whether the current species descriptions are valid as far as morphometrical characters are concerned. Usually mean and standard deviation of these characters in the analyzed 'populations' are given, but their partly high correlation is not considered. Therefore we made a proportion analysis. Ignoring this correlation might bias comparisons.

The specimens from the Lobau can be seen as a transition, *i.e.* they differ very much from those of Orth/Danube; this leads to the impression that there was a long lasting isolated evolution of both groups. Of course, the reasons for the metrical differences may not be genetic at all, but may be adaption to ecological factors.

For *L. attenuatus*, the situation is even more complex, because the individuals of the Lobau (area V) are quite different from those of area IV (Orth/Danube). They can almost perfectly be separated from each other. There is no great distance between these areas, neither in a geographical, nor in an ecological sense, so it is difficult to explain this result.

In Fig. 4, *Longidorus leptocephalus* from arable land and *L. intermedius*, mainly from area I, are used as outgroups and are laying at the periphery of the hemisphere, as well as *L. attenuatus* from area IV and area I. Using the nearest neighbour procedure, we made sure, that the specimens of *L. attenuatus* from area V (in the centre of Fig. 4) are clustering together and therefore are a homogenous group. There is just a small overlap between the area IV (and area I and II) and the area V 'population'.

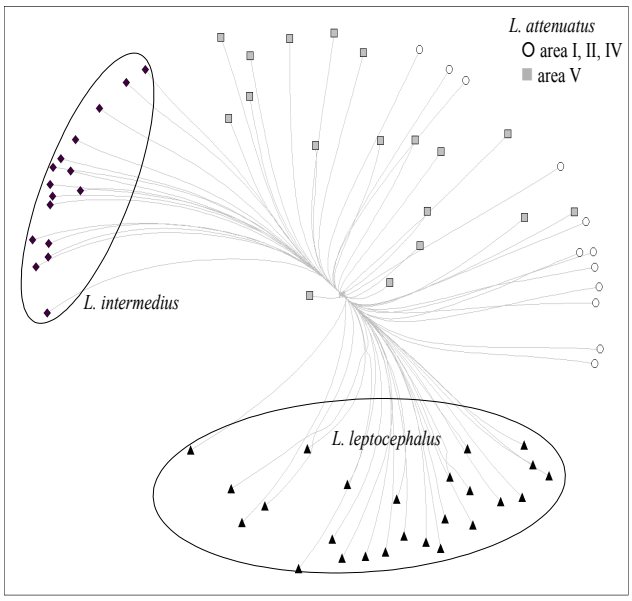

Fig. 4: Morphometrical comparison of two local 'populations' of *Longidorus attenuatus, L. leptocephalus* and *L. intermedius* are used as outgroups.

Using the key of CHEN *et al*. (1997), for the individuals from area V we get the code: A3, B2, C2, D34, E2, F2, G2, H4, I1, that differs from the one published for *L. attenuatus* only in F (*L. attenuatus* is F34). Hence, the area V specimens are unusually short (the F-code concerns body length). The animals of area IV have the typical code for *L. attenuatus*.

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References

ARNOLD, C.; GILLET, F.; GOBAT, J. M.; 1998: Situation de la vigne sauvage *Vitis vinifera* spp. *silvestris* en Europe. Vitis **37**, 159-170.

BOWERS, J. E., DANGL, G. S.; MEREDITH, C. P.; 1999: Development and characterization of additional microsatellite DNA markers for grape. Amer. J. Enol. Vitic. **50**, 243-246

BOWERS, J. E.; DANGL, G. S.; VIGNANI, R.; MEREDITH, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome **39**, 628-633.

CHEN, Q.; HOOPER, D. J.; LOOF, P. A. A.; XU, J.; 1997: A revised polytomous key for the identification of species of the genus Longidorus Micoletzky, 1922 (*Nematoda: Dorylaimoidea*), Fundam. Appl. Nematol. **20**, 15-28.

GANGL, H.; LEITNER, G.; TIEFENBRUNNER, W.; 2001: Rebschädigende Viren, Bakterien und bodenbürtige Vektoren im österreichischen Weinbaugebiet Carnuntum, Mitt. Klosterneuburg **51**, 123-132.

JACQUIN, N. J.; 1792: *Enumeratio stirpium plerarumque, quae sponte crescunt in agro vindobonensi, montibusque confinibus*, Wien.

KIRCHHEIMER, F.; 1955: Über das Vorkommen der wilden Weinrebe in Niederösterreich und Mähren, Z. Bot. **43**, 279-307.

LOOF, P. A. A.; CHEN, Q.; 1999: A revised polytomous key for the identification of species of the genus Longidorus Micoletzky, 1922 (*Nematoda: Dorylaimoidea*). Nematology **1**, 55-59.

LOOF, P. A. A.; LUC, M.; 1990: A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (*Nematoda: Longidoridae*) with exclusion of the *X. americanum* group. Syst. Parasitol. **16**, 35-66.

LOOF, P. A. A.; LUC, M.; 1993: A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (*Nematoda: Longidoridae*) with exclusion of the *X. americanum* group, suppl. 1. Syst.c Parasitol. **24**, 185-189.

LOOF, P. A. A.; LUC, M.; 1996: A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (*Nematoda: Longidoridae*) with exclusion of the *X. americanum* group, suppl. 2. Syst. Parasitol. **33**, 23-29.

NEI, M.; 1972: Genetic distance between populations, Am. Naturalist **106**, 183-191.

REGNER, F.; STADLBAUER, A.; EISENHELD, C.; 1998: Heunisch x Fränkisch, ein wichtiger Genpool europäischer Rebsorten (*Vitis vinifera* L *sativa*). Vitic. Enol. Sci. **53**, 114-118.

REGNER, F.; STADLBAUER, A.; EISENHELD, C.; KASERER, H.; 2000: Genetic Relationship among Pinots and related cultivars. Am. J. Enol. Vitic. **51**, 7-14.

REGNER, F.; STADLBAUER, A.; KASERER, H.; EISENHELD, C.; 2001: Weitere Sortenanalysen bei Reben mittels genetischer Marker. Mitt. Klosterneuburg **51**, 3-14.

SEFC, K. M.; REGNER, F.; TURETSCHEK, E.; GLOESSL, J.; STEINKELLNER, H.;1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability to genotype different *Vitis* species. Genome **42**, 1-7.

SCHULZ, T. F.; LORENZ, D.; EICHHORN, K. W.; OTTEN, L.; 1993: Amplification of different marker sequences for identification of *Agrobacterium vitis* strains, Vitis **32**, 179-182.

THOMAS, M. R.; MATSUMOTO, S., CAIN, P.; SCOTT, N. S. 1993: Repetitive DNA of grapevine: Classes present and sequences suitable for cultivar identification Theor. Appl. Genet. **86**, 173-180.

Thomas, M. R.; Scott, N.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor. Appl. Genet. **86**, 985-990.

TIEFENBRUNNER, A.; TIEFENBRUNNER, M.; TIEFENBRUNNER, W.; WAHRA, A.; 2002: A software tool as an aid to the identification of species of Longidorus Micoletzky, 1922 (*Nematoda: Dorylaimoidea*), Nematology **4**, 845-852.

TIEFENBRUNNER, W.; REGNER, F.; MANDL, K.; LEITNER, G.; GANGL, H.; 2004 a: The wild vine (*Vitis vinifera ssp. silvestris*) in the riparian woods of Donau and March (Austria): Evaluation of genetic divergence, potential on grape damaging viruses, bacterials and soil-borne vectors, Plant Genet. Res. Newsletter, in press.

TIEFENBRUNNER, A.; TIEFENBRUNNER, W.; 2004 b: *Longidoridae* (*Nematoda: Dorylaimida*) from the rhizosphere of the wild growing grape (*Vitis vinifera* ssp. *silvestris*) in the riparian woods of the rivers Danube and March in Austria. Helminthologia **41**, 45-53.

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